APPLICATION IN

THE UNITED STATES
PATENT AND TRADEMARK OFFICE

FOR

COMPOSITION AND METHOD FOR INHIBITING PLATELET AGGREGATION

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COMPOSITION AND METHOD FOR INHIBITING PLATELET AGGREGATION

This application is a continuation-in-part of U.S. Application No. 09/934,970, filed August 21, 2001, which is a continuation-in-part of U.S. Application No. 09/643,138, filed August 21, 2000. The contents of both applications are incorporated herein by reference in their entirety.

TECHNICAL FIELD

This invention relates to mono- and dinucleoside polyphosphate compounds and the method of using such compounds in the prevention or treatment of diseases or conditions associated with platelet aggregation, including thrombosis in humans and other mammals.

BACKGROUND OF THE INVENTION

Hemostasis is the spontaneous process of stopping bleeding from damaged blood vessels. Precapillary vessels contract immediately when cut; within seconds, thrombocytes, or blood platelets, are bound to the exposed matrix of the injured vessel by a process called platelet adhesion. Platelets also stick to each other in a phenomenon known as platelet aggregation to form a platelet plug to stop bleeding quickly.

An intravascular thrombus results from a pathological disturbance of hemostasis. Platelet adhesion and aggregation are critical events in intravascular thrombosis. Activated under conditions of turbulent blood flow in diseased vessels or by the release of mediators from other circulating cells and damaged endothelial cells lining the vessel, platelets accumulate at a site of vessel injury and recruit further platelets into the developing thrombus. The thrombus can grow to sufficient size to block off arterial blood vessels. Thrombi can also form in areas of stasis or slow blood flow in veins. Venous thrombi can easily detach portions of themselves called emboli that travel through the circulatory system and can result in blockade of other vessels, such as pulmonary arteries. Thus, arterial thrombi cause serious disease by local blockade, whereas venous thrombi do so primarily by distant blockade, or embolization. These conditions include venous thrombosis, thrombophlebitis, arterial embolism, coronary and cerebral arterial thrombosis, unstable angina, myocardial infarction, stroke, cerebral embolism, kidney embolisms and pulmonary embolisms.

A number of converging pathways lead to platelet aggregation. Whatever the initial stimulus, the final common event is crosslinking of platelets by binding fibrinogen to a membrane binding site, glycoprotein IIb/IIIa (GPIIb/IIIa). Compounds that are antagonists for

GPIIb/IIIa receptor complex have been shown to inhibit platelet aggregation (U.S. Patent Nos. 6,037,343 and 6,040,317). Antibodies against GPIIb/IIIa have also been shown to have high antiplatelet efficacy (The EPIC investigators, *New Engl. J. Med.* (1994) 330:956-961). However, this class of antiplatelet agents sometimes causes bleeding problems.

Thrombin can produce platelet aggregation largely independently of other pathways but substantial quantities of thrombin are unlikely to be present without prior activation of platelets by other mechanisms. Thrombin inhibitors such as hirudin are highly effective antithrombotic agents. However, functioning as both antiplatelet and anti-coagulant agents, thrombin inhibitors again can produce excessive bleeding. (The TIMI 9a investigators, The GUSTO Iia investigators, *Circulation*, 90: 1624-1630 (1994); *Circulation*, 90: 1631-1637 (1994); Neuhaus K. L. *et al.*, *Circulation*, 90: 1638-1642 (1994))

Various antiplatelet agents have been studied for many years as potential targets for inhibiting thrombus formation. Some agents such as aspirin and dipyridamole have come into use as prophylactic antithrombotic agents, and others have been the subjects of clinical investigations. To date, the powerful agents such as disintegrins, and the thienopyridines ticlopidine and clopidogrel have been shown to have substantial side effects, while agents such as aspirin have useful but limited effectiveness (Hass, *et al.*, *N. Engl. J. Med.*, 321:501-507 (1989); Weber, *et al.*, *Am. J. Cardiol.* 66:1461-1468 (1990); Lekstrom and Bell, *Medicine* 70:161-177 (1991)). In particular, use of the thienopyridines in antiplatelet therapy has been shown to increase the incidence of potentially life threatening thrombotic thrombocytopenic purpura (Bennett, C.L. *et al. N. Engl. J. Med*, (2000) 342: 1771-1777). Aspirin, which has a beneficial effect on platelet aggregation (*Br. Med. J.* (1994) 308: 81-106; 159-168), acts by inducing blockade of prostaglandin synthesis. Aspirin has no effect on ADP-induced platelet aggregation, and thus has limited effectiveness on platelet aggregation. Furthermore, its well documented high incidence of gastric side effects limits its usefulness in many patients. Clinical efficacy of some newer drugs, such as ReoPro (7E3), is impressive, but recent trials have found that these approaches are associated with an increased risk of major bleeding, sometimes necessitating blood transfusion (*New Engl. J. Med.* (1994) 330:956-961). Thus it appears that the ideal "benefit/risk" ratio has not been achieved.

Recent studies have suggested that adenosine 5'-diphosphate (ADP), a common agonist, plays a key role in the initiation and progression of arterial thrombus formation (Bernat, *et al.*, *Thromb. Haemostas.* (1993) 70:812-826); Maffrand, *et al.*, *Thromb. Haemostas.* (1988) 59:225-230; Herbert, *et al.*, *Arterioscl. Thromb.* (1993) 13:1171-1179).

ADP induces platelet aggregation, shape change, secretion, influx and intracellular mobilization of Ca^{+2}, and inhibition of adenylyl cyclase. Binding of ADP to platelet receptors is required for elicitation of the ADP-induced platelet responses. There are at least three P2 receptors expressed in human platelets: a cation channel receptor $P2X_1$, a G protein-coupled receptor $P2Y_1$, and a G protein-coupled receptor $P2Y_{12}$ (also referred to as $P2Y_{ac}$ and $P2_T$). The $P2X_1$ receptor is responsible for rapid calcium influx and is activated by ATP and by ADP. However, its direct role in the process of platelet aggregation is unclear. The $P2Y_1$ receptor is responsible for calcium mobilization, shape change and the initiation of aggregation. $P2Y_{12}$ receptor is responsible for inhibition of adenylyl cyclase and is required for full aggregation. (Hourani, *et al.*, The Platelet ADP Receptors Meeting, La Thuile, Italy, March 29-31, 2000)

Ingall *et al.* (*J. Med. Chem.* 42: 213-220, (1999)) describe a dose-related inhibition of ADP-induced platelet aggregation by analogues of adenosine triphosphate (ATP), which is a weak, nonselective but competitive $P2Y_{12}$ receptor antagonist. Zamecnik (USPN 5,049,550) discloses a method for inhibiting platelet aggregation in a mammal by administering to said mammal a diadenosine tetraphosphate compound of App(CH_2)ppA or its analogs. Kim *et al.* (USPN 5,681,823) disclose P^1, P^4-dithio-P^2, P^3-monochloromethylene 5', 5''' diadenosine P^1, P^4-tetraphosphate as an antithrombotic agent. The thienopyridines ticlopidine and clopidogrel, which are metabolized to antagonists of the platelet $P2Y_{12}$ receptor, are shown to inhibit platelet function *in vivo* (Quinn and Fitzgerald, *Circulation* 100:1667-1672 (1999); Geiger, *et al.*, *Arterioscler. Thromb. Vasc. Biol.* 19:2007-2011 (1999)).

There is a need in the area of cardiovascular and cerebrovascular therapeutics for an agent that can be used in the prevention and treatment of thrombi, with minimal side effects, such as unwanted prolongation of bleeding, while preventing or treating target thrombi.

SUMMARY OF THE INVENTION

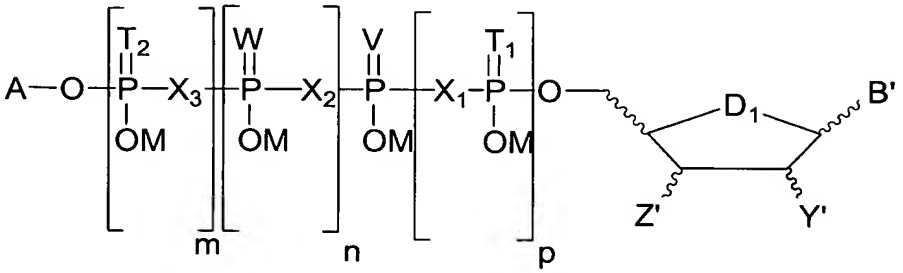
This invention is directed to a method of preventing or treating diseases or conditions associated with platelet aggregation; such diseases include venous thrombosis, thrombophlebitis, arterial embolism, coronary and cerebral arterial thrombosis, unstable angina, myocardial infarction, stroke, cerebral embolism, kidney embolisms and pulmonary embolisms. The method is also directed to a method of preventing, treating or reducing the incidence of: thrombosis, thrombotic events, embolic events or pathological conditions

associated with such events, where the thrombosis, thrombotic event or embolic event occurs during or after surgery.

The method comprises administering to a subject a pharmaceutical composition comprising a therapeutic effective amount of $P2Y_{12}$ receptor antagonist compound, wherein said amount is effective to bind the $P2Y_{12}$ receptors on platelets and inhibit ADP-induced platelet aggregation.

The $P2Y_{12}$ receptor antagonist compounds useful for this invention include compounds of general Formula I, or salts thereof:

Formula I



wherein:

X_1, X_2, and X_3 are independently oxygen, methylene, monochloromethylene, dichloromethylene, monofluoromethylene, difluoromethylene, or imido;

T_1, T_2, W, and V are independently oxygen or sulfur;

m = 0,1 or 2;

n = 0 or 1;

p = 0,1, or 2 ;

where the sum of m+n+p is from 0 to 5; (monophosphate to hexaphosphate)

M = H or a pharmaceutically-acceptable inorganic or organic counterion;

D_1 =O or CH_2;

B' is a purine or a pyrimidine residue according to general Formulae IV and V which is linked to the 1' position of the furanose or carbocycle via the 9- or 1- position of the base, respectively;

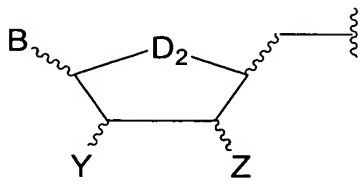
Y' = H, OH, or OR_1;

Z' = H, OH, or OR_2;

with the proviso that when A = M, at least one of Y' and Z' is equal to OR_1 or OR_2 respectively;

A = M, or

A is a nucleoside residue which is defined as:



and which is linked to the phosphate chain via the 5' position of the furanose or carbocycle; wherein:

D_2 = O or CH_2;

Z = H, OH, or OR_3;

Y = H, OH, or OR_4;

with the proviso that at least one of Y', Z', Y and Z is equal to OR_1, OR_2 OR_3 or OR_4 respectively;

B is a purine or a pyrimidine residue according to general Formulae IV and V which is linked to the 1'- position of the furanose or carbocycle via the 9- or 1- position of the base, respectively;

R_1, R_2, R_3, and/or R_4 are residues which are linked directly to the 2'- and /or 3'- hydroxyls of the respective furanose or carbocycle via a carbon atom according to Formula II, or linked directly to two (2'- and 3'-) hydroxyls of the respective furanose or carbocycle via a common carbon atom according to Formula III, such that from one to four independent residues of R_1, R_2, R_3 and R_4 falling within the definition of Formula II are present or from one to two independent residues made up of R_1+R_2 and/or R_3+R_4 are present.

The present invention also provides novel mononucleoside 5'-monophosphate-, mononucleoside polyphosphate-, and dinucleoside polyphosphate-compounds which are useful in this invention. The present invention further provides pharmaceutical formulations comprising mononucleoside 5'-monophosphates, mononucleoside polyphosphates, or dinucleoside polyphosphates.

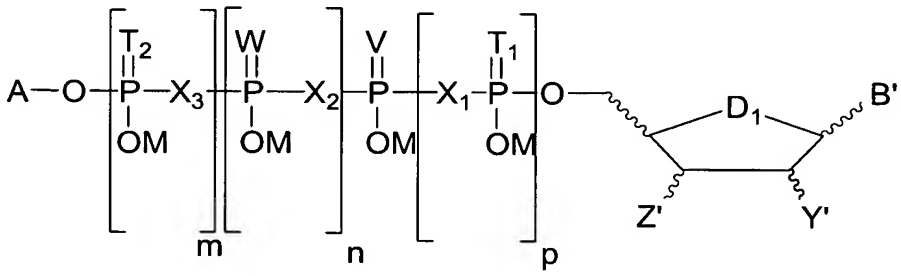
BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the effect of inhibition of ADP-induced aggregation by different compounds.

DETAILED DESCRIPTION OF THE INVENTION

This invention is provides a method of preventing or treating diseases or conditions associated with platelet aggregation. The method also provides a method of treating thrombosis. The method comprises administering to a subject a pharmaceutical composition comprising a therapeutic effective amount of $P2Y_{12}$ receptor antagonist compound, wherein said amount is effective to bind the $P2Y_{12}$ receptors on platelets and inhibit ADP-induced platelet aggregation. The $P2Y_{12}$ receptor antagonist compounds useful for this invention include compound of general Formula I and salts thereof:

Formula I



wherein:

X_1, X_2, and X_3 are independently oxygen, methylene, monochloromethylene, dichloromethylene, monofluoromethylene, difluoromethylene, or imido;

T_1, T_2, W, and V are independently oxygen or sulfur;

m = 0, 1 or 2;

n = 0 or 1;

p = 0, 1, or 2 ;

where the sum of m + n + p is from 0 to 5; (from monophosphate to hexaphosphate)

M = H, or a pharmaceutically-acceptable inorganic or organic counterion;

D_1 = O or CH_2

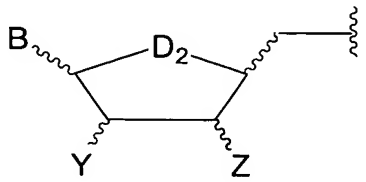
B' is a purine or a pyrimidine residue according to general Formulae IV and V which is linked to the 1'- position of the furanose or carbocycle via the 9- or 1- position of the base, respectively;

Y' = H, OH, or OR_1;

Z' = H, OH, or OR_2; with the proviso that when A = M, at least one of Y' and Z' is OR_1 or OR_2;

A = M, or

A is a nucleoside residue which is defined as:



and which is linked to the phosphate chain via the 5'- position of the furanose or carbocycle; wherein:

D_2 = O or CH_2;

Z = H, OH, or OR_3;

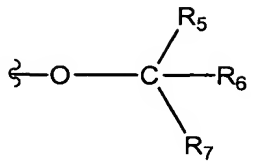
Y = H, OH, or OR_4;

with the proviso that at least one of Y', Z', Y and Z is equal to OR_1, OR_2 OR_4 or OR_3 respectively.

B is a purine or a pyrimidine residue according to general Formulae IV and V which is linked to the 1' position of the furanose or carbocycle via the 9- or 1- position of the base, respectively;

R_1, R_2, R_3, and/or R_4 are residues which are linked directly to the 2'- and /or 3'- hydroxyls of the respective furanose or carbocycle via a carbon atom according to Formula II, or linked directly to two (2'- and 3'-) hydroxyls of the respective furanose or carbocycle via a common carbon atom according to Formula III, such that from one to four independent residues of R_1, R_2, R_3 and R_4 falling within the definition of Formula II are present or from one to two independent residues made up of R_1+R_2 and/or R_3+R_4 are present;

Formula II



wherein:

O is the corresponding 2'- and/or 3'- oxygen of the respective furanose or carbocycle;

C is a carbon atom;

R_5, R_6, and R_7 are H, alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, such that the moiety defined according to Formula II is an ether; or

R_5 and R_6 are H, an alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, and R_7 is alkoxy, cycloalkoxy, aralkyloxy, aryloxy, substituted aralkyloxy, or substituted aryloxy such that the moiety defined according to Formula II is an acyclic acetal or ketal; or

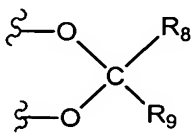
R_5 and R_6 are taken together as oxygen or sulfur doubly bonded to C, and R_7 is alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, such that the moiety defined according to Formula II is an ester or thioester; or

R_5 and R_6 are taken together as oxygen or sulfur doubly bonded to C, and R_7 is amino or mono- or disubstituted amino, where the substituents are alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, such that the moiety according to Formula II is a carbamate or thiocarbamate; or

R_5 and R_6 are taken together as oxygen or sulfur doubly bonded to C, and R_7 is alkoxy, cycloalkoxy, aralkyloxy, aryloxy, substituted aralkyloxy, or substituted aryloxy, such that the moiety according to Formula II is a carbonate or thiocarbonate; or

R_7 is not present and R_5 and R_6 are taken together as oxygen or sulfur doubly bonded to C and both the 2'- and 3'- oxygens of the respective furanose or carbocycle are directly bound to C to form a cyclical carbonate or thiocarbonate;

Formula III



wherein:

the O atoms are the 2'- and 3'- oxygens of a furanose or carbocycle; and the 2'- and 3'- oxygens of the furanose or carbocycle are linked by a common carbon atom (C) to form a cyclical acetal, cyclical ketal, or cyclical orthoester;

for cyclical acetals and ketals, R_8 and R_9 are independently hydrogen, alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, substituted aryl, or can be joined together to form a homocyclic or heterocyclic ring composed of 3 to 8 atoms, preferably 3 to 6 atoms;

for cyclical orthoesters, R_8 is hydrogen, alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl, or substituted aryl, R_9 is alkyloxy, cycloalkyloxy, aralkyloxy, aryloxy, substituted aralkyloxy, or substituted aryloxy.

when present, the alkyl, cycloalkyl, aralkyl, aryl, substituted aralkyl and substituted aryl components of R_5 to R_9 can be generally defined as, but are not limited to, the following:

alkyl groups are from 1 to 12 carbons inclusively, either straight chained or branched, with or without unsaturation and with or without heteroatoms, are more preferably from 2 to 8 carbons inclusively, and most preferably 2 to 6 carbons inclusively;

cycloalkyl groups from 3 to 12 carbons inclusively, more preferably from 3 to 10 carbons inclusively, and most preferably 3 to 6 carbons inclusively, with or without unsaturation, and with or without heteroatoms;

aralkyl groups are from 1 to 8 carbons inclusively in the alkyl portion, are more preferably from 1 to 6 carbons inclusively in the alkyl portion, and most preferably are 1 to 4 carbons inclusively in the alkyl portion; as included in the alkyl definition above, the alkyl portion of an aralkyl group can include one or more positions of unsaturation such as a double bonds or a triple bond in the chain when the chain includes two or more carbon atoms; the alkyl portion of an aralkyl group can also include one or more heteroatoms and/or substituents; the aryl portion of an aralkyl group can be a monocyclic or polycyclic moiety from 3 to 8 carbons inclusively per ring in the aryl portion, more preferably from 4 to 6 carbons inclusively per ring, and most preferably 5 to 6 carbons inclusively per ring; the aryl portion of an aralkyl group can can also bear one or more substituents and/or heteroatoms;

aryl groups are either monocyclic or polycyclic, are from 3 to 8 carbons inclusively per ring, are more preferably from 4 to 6 carbons inclusively per ring, and are most preferably 5 to 6 carbons inclusively per ring; aryl groups can also bear substituents and/or heteroatoms.

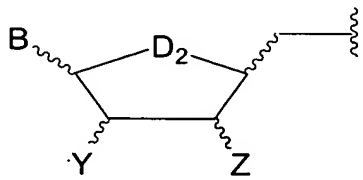
Preferred substituents on the foregoing groups can be, but are not limited to, hydroxy, nitro, methoxy, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, thioalkyl, alkoxy, carboxyl, cyano, amino, substituted amino, trifluoromethyl, phenyl, cyclopropyl, cyclopentyl, and cyclohexyl; and preferred heteroatoms are oxygen, nitrogen, and sulfur.

When R_5, R_6 and R_7 are not the same, or when R_8 and R_9 are not the same, a compound according to Formula I can exist in several diastereomeric forms. The general structure of Formula I includes all diastereomeric forms of such materials, when not specified otherwise. Formula I also includes mixtures of compounds of Formula I, including mixtures of enantiomers, diastereomers and/or other isomers in any proportion.

One embodiment of the invention is that A= M, wherein M =H or a pharmaceutically-acceptable inorganic or organic counterion. In such an embodiment, the compound can be a nucleoside monophosphate, nucleoside diphosphate, nucleoside triphosphate, nucleoside tetraphosphate, nucleoside pentaphosphate, or nucleoside hexaphosphate with one or both of the 2'- and/or 3'- positions of the furanose or carbocycle modified. Most preferred are nucleotide monophosphates, nucleotide diphosphates, nucleotide triphosphates, and nucleotide tetraphosphates. When T_2, W, V, or T_1 are sulfur, the preferred position for this atom is on the terminal phosphorous of the polyphosphate chain (i.e. the phosphorous furthest removed from the nucleoside residue).

For monophosphates, where m, n, and p are all equal to zero, preferably R_8 is hydrogen and R_9 is aryl or aralkyl, with 1, 2, 3, or 4 carbons inclusively in the alkyl portion of an aralkyl group, and 6 carbons inclusively in the aryl portion of an aralkyl or aryl group; when the number of carbons in the alkyl portion of an aralkyl group is 2, the carbon atoms are most preferably connected by either a double or triple bond.

Another embodiment of the invention is that A is a nucleoside residue defined as:



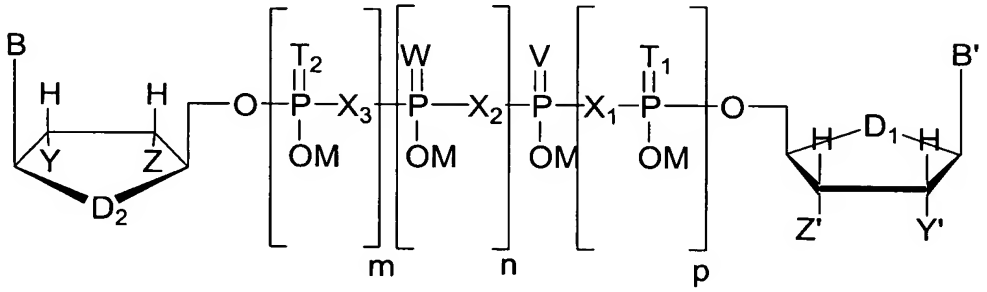
and linked to the phosphate chain via the 5'- position of the furanose or carbocycle (to give a dinucleoside polyphosphate with at least one of the 2'-, 3'-, 2''- and 3''- positions of the furanose or carbocycle moieties modified to be OR_1, OR_2, OR_4 or OR_3 respectively).

When T_2, W, V, and/or T_1 are sulfur, the preferred positions (for sulfur) are T_1 and T_2.

Further provisions are that when either D_1 or D_2 are oxygen, the corresponding furanose is preferably in the β-configuration; and that the corresponding furanose is most preferably in the β-D-configuration.

Preferred compounds of general Formula I are molecules whose structures fall within the definitions of Formula Ia and Formula Ib:

Formula Ia



wherein:

D_1 = O or CH_2;

D_2 = O or CH_2;

B and B' are independently purine or pyrimidine residues according to general Formula IV or V;

m and p = 0, 1 or 2; n = 0 or 1; such that the sum of m + n + p is from 0 to 5, preferably 0 to 4, and most preferably 0 to 3;

X_1, X_2, and X_3 = are independently O, NH, CH_2, CHF, CHCl, CF_2, CCl_2;

T_1, T_2, V, and W are independently O or S;

M = H^+, NH_4^+, Na^+ or other pharmaceutically-acceptable inorganic or organic counter ion;

Y'= H, OH, or OR_1;

Z' = OH or OR_2;

Z = OH or OR_3;

Y = H, OH, or OR_4, where R_1, R_2, R_3 and R_4 fall under the definition of general Formulae II or III, provided that at least one of Y', Z', Z and Y is OR_1, OR_2, OR_3, or OR_4.

Preferred compounds of Formula Ia include:

D_1 = O or CH_2;

D_2 = O or CH_2;

X_1, X_2, and X_3 =O;

T_1, T_2, V, and W = O; or

D_1 = O or CH_2;

D_2 = O or CH_2;

X_1 and X_3 = O;

X_2 = methylene, monochloromethylene, dichloromethylene, monofluoromethylene, difluoromethylene, or imido;

T, T_1, T_2, V, and W = O; or

D_1 = O or CH_2;

D_2 = O or CH_2;

m, n, and p = 1; or

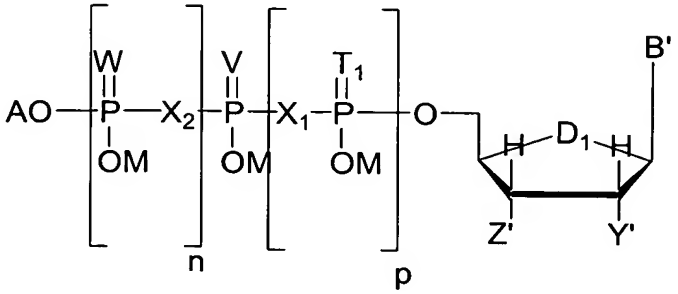
X_1 and X_3 = O;

X_2 = methylene, monochloromethylene, dichloromethylene, monofluoromethylene, difluoromethylene, or imido;

T_1 and T_2 = S;

V and W = O.

Formula Ib



D_1 = O or CH_2;

n and p = 0, 1, or 2 such that the sum of n + p is from 0 to 3;

A = M; wherein M = H^+, NH_4^+, Na^+ or other pharmaceutically-acceptable inorganic or organic counterion;

B' is a purine or pyrimidine residue according to general Formulae IV and V;

X_1 and X_2 are independently O, NH, CH_2, CHF, CHCl, CF_2, CCl_2;

T_1, V, and W are independently O or S;

Y' = H, OH, or OR_1,

Z' = H, OH or OR_2, where R_1 and R_2 fall under the definitions of general Formulae II or III; with the proviso that at least one of Y' and Z' is OR_1 or OR_2, respectively.

Preferred compounds of Formula Ib include:

D_1 = O or CH_2;

n and p = 0, 1, or 2 such that the sum of n+p is from 0 to 3, preferably 1 to 2;

X_1 and X_2 = O;

T_1, V, and W = O; or

D_1 = O or CH_2;

X_1 and X_2 = O;

T_1 and V = O;

W = S; or

D_1 = O;

n and p = 0 such that the sum of n + p is 0;

V= O;

B' is a purine residue of general Formula IV;

Y' and Z' fall under the definition of general Formula III; or

D_1 = O or CH_2;

p = 0, 1, or 2, n = 1, such that the sum of n + p is from 1 to 3;

X_1 = O;

X_2 = methylene, monochloromethylene, dichloromethylene, monofluoromethylene, difluoromethylene, or imido;

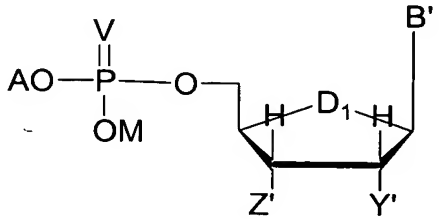
T_1, V, and W = O;

Y' = H, OH, or OR_1;

Z' = H, OH or OR_2, where R_1 and R_2 fall under the definition of general Formulae II or III; with the proviso that at least one of Y' and Z' is OR_1 or OR_2, respectively.

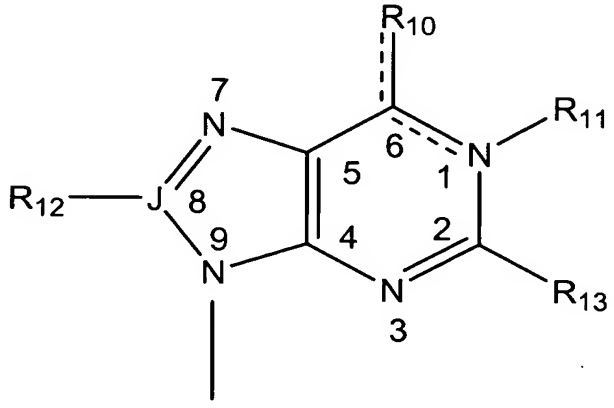
Several preferred compounds also are described by Formula Ib':

Formula Ib'

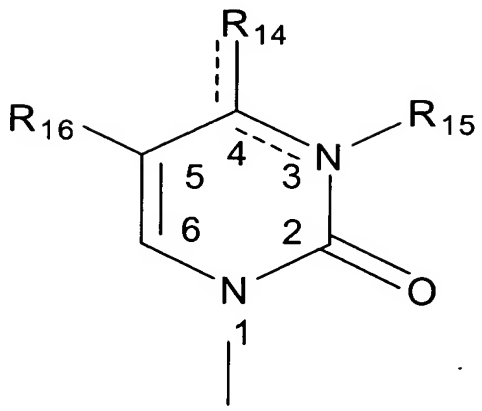


For compounds of Formula I, B and B' can independently be a purine residue, as in Formula IV, linked through the 9- position, or a pyrimidine residue, as in Formula V, linked through the 1- position. The ribosyl moieties in Formulae Ia, Ib, and Ib' are in the D-configuration as shown, but can also be L-, or D- and L-. The D- configuration is preferred for ribosyl moieties.

Formula IV



Formula V



wherein:

R_{10} and R_{14} independently are hydroxy, oxo, amino, mercapto, alkylthio, alkyloxy, aryloxy, alkylamino, cycloalkylamino, aralkylamino, arylamino, diaralkylamino, diarylamino, or dialkylamino, where the alkyl groups are optionally linked to form a heterocycle; or

R_{10} and R_{14} independently are acylamino, provided that they incorporate an amino residue from the C-6 position of the purine or the C-4 position of the pyrimidine; or

when R_{10} in a purine or R_{14} in a pyrimidine has as its first atom nitrogen, R_{10} and R_{11} or R_{14} and R_{15} can be taken together to form a 5-membered fused imidazole ring (to give an etheno compound), optionally substituted on the etheno ring with one or more alkyl, cycloalkyl, aralkyl, or aryl moieties, as described for R_5-R_9 above;

J is carbon or nitrogen, with the provision that when J = nitrogen, R_{12} is not present;

R_{11} is hydrogen, O (adenine 1-oxide derivatives) or is absent (adenine derivatives);

R_{15} is hydrogen, or acyl (e.g. acetyl, benzoyl, phenylacyl, with or without substituents);

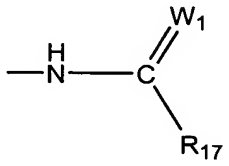
R_{12} is hydrogen, alkyl, bromo, azido, alkylamino, arylamino or aralkylamino, alkoxy, aryloxy or aralkyloxy, alkylthio, arythio or aralkylthio, or ω-A(C_{1-6}alkyl)B-, wherein A and B are independently amino, mercapto, hydroxy or carboxyl;

R_{13} is hydrogen, chlorine, amino, monosubstituted amino, disubstituted amino, alkylthio, arylthio, or aralkylthio, where the substituent on sulfur contains up to a maximum of 20 carbon atoms, with or without unsaturation, and with or without substituents on the chain;

R_{16} is hydrogen, methyl, alkyl, halogen, alkyl, alkenyl, substituted alkenyl, alkynyl, or substituted alkynyl.

Compounds according to Formulae IV and V where R_{10} or R_{14} is acylamino fall within the scope of Formula VI:

Formula VI



wherein:

NH is the amino residue at the C-6 position in a purine or the amino residue at the C-4 position in a pyrimidine;

C is a carbon atom;

W_1 is oxygen or sulfur;

R_{17} is amino or mono- or disubstituted amino, with the amino substituent(s) being alkyl, cycloalkyl, aralkyl, or aryl, with or without further substituents, unsaturation, or heteroatoms, such that the moiety according to Formula VI is a urea or thiourea; or

R_{17} is alkoxy, aralkyloxy, aryloxy, substituted aralkyloxy, or substituted aryloxy, such that the moiety according to Formula VI is a carbamate or thiocarbamate; or

R_{17} is alkyl, cycloalkyl, aralkyl, or aryl, with or without substituents or heteroatoms, such that the moiety according to Formula VI is an amide; with definitions of alkyl, cycloalkyl, aralkyl, or aryl groups as previously defined for comparable groups in R_5 to R_9.

The compounds of the present invention can be conveniently synthesized by those skilled in the art using well-known chemical procedures. Mononucloside mono-, di- and triphosphates can be obtained from commercial sources or can be synthesized from the nucleoside using a variety of phosphorylation reactions which can be found in the chemical literature. Symmetrical and unsymmetrical dinucleotide polyphosphates can be prepared by activation of a nucleoside mono-, di- or triphosphate with a coupling agent such as, but not limited to, dicyclohexylcarbodiimide or 1, 1'-carbonyldiimidazole, followed by condensation with another nucleoside mono-, di-, or triphosphate, which can be the same or different as the

activated moiety. Activation of nucleoside triphosphates with dicyclohexylcarbodiimide gives a cyclical trimetaphosphate as the activated species, which can be advantageously reacted with a variety of nucleophiles to install unique substituents on the terminal phosphate of a triphosphate.

The compounds of the present invention can be prepared by derivatization or substitution at the level of the nucleoside, followed by phosphorylation and condensation as previously described, or the reactions can be carried out directly on the preformed mono- or dinucleotides. In the general Formulae Ia and Ib, the substituents at Y', Z', Y, and Z can be esters, carbamates, or carbonates, which are generally described by Formula II. Esters can be readily prepared by reacting a hydroxyl group of the furanose in a nucleoside or nucleotide with an activated form of an appropriate organic acid, such as an acid halide or acid anyhydride in the presence of an organic or inorganic base. Alternately, use of a suitable coupling reagent such as dicyclohexylcarbodiimide, 1,1'- carbonyldiimidazole and the like to activate the organic acid can be used to achieve the same result.

Carbamates or thiocarbamates can be most conveniently prepared by reaction of a hydroxyl group of the furanose in a nucleoside or nucleotide with any of a number of commercially available isocyanates or isothiocyanates, respectively, in an inert solvent. Alternately, when a desired isocyanate or isothiocyanate cannot be obtained from commercial sources, it can be prepared from the corresponding amine by the use of phosgene or thiophosgene, respectively, or their chemical equivalents. Carbonates or thiocarbonates can be synthesized by reacting the hydroxyl groups of a furanose in a nucleoside or nucleotide with an appropriate haloformate in the presence of an organic or inorganic base.

In the general Formulae Ia, Ib and Ib', the substituents at Y'and Z', and Y and Z, when taken together, can be taken to mean acetals, ketals or orthoesters, as described in Formula III. Acetals and ketals can be readily prepared by reaction of the neighboring 2'- and 3'- hydroxyl groups of the furanose in an appropriate nucleoside or nucleotide with an aldehyde or ketone, respectively, or their chemical equivalents, in the presence of an acid catalyst. Particularly advantageous is the use of an organic acid, which can effect the transformation without affecting the integrity of the rest of the molecule. Alternately, strong acids such as trichloroacetic, p-toluenesulfonic, methanesulfonic and the like can be employed in catalytic amounts, in conjunction with inert solvents. Most preferred is formic acid, which can be removed by evaporation under reduced pressure, and is ideally suited to serve as both solvent and catalyst for these reactions. Alternately, trifluoroacetic acid can be substituted for formic

acid in the reaction, provided that the reaction is carried out at low temperatures and the aldehyde or aldehyde equivalent used to prepare the acetal is stable to strong acid conditions. The discovery of the utility of formic acid and trifluoroacetic acid for this purpose is one particular aspect of this invention.

Cyclical orthoesters can be prepared by reaction of the neighboring 2'- and 3'-hydroxyl groups of a furanose with an acylic orthoester, in the presence of an acid. When the nucleoside or nucleotide to be derivatized is a purine that contains a 6-amino functionality or is a pyrimidine that contains a 4-amino functionality, it can be converted to the respective urea or thiourea by treatment with an isocyanate or isothiocyanate, respectively, as was previously described for carbamates or thiocarbamates of the 2'- or 3'- hydroxyls of a furanose. It was found that reactions of such an amino group with isocyanates or isothiocyanates can be carried out in the presence of one or more hydroxyl groups on a furanose, by appropriate manipulation of the stoichiometry of the reaction.

All of the derivitization reactions described herein can be carried out on preformed dinucleotide polyphosphates, which results in multiple products dependent on reaction stoichiometry and on whether multiple reactive groups are present. When multiple products are obtained, these can be conveniently separated by the use of preparative reverse phase high performance liquid chromatography (HPLC). Particularly advantageous is the use of C18 or phenyl reverse phase columns, in conjunction with gradients that start with ammonium acetate buffer and end with methanol. The use of a buffer provides for nucleotide stability and improved peak shape of the eluting products and the use of methanol allows for effective desorption of these lipophilic compounds from the column. Particularly advantageous is the use of ammonium acetate buffer solutions in conjunction with methanol, as these solvents are miscible in all proportions and can be readily removed from the chromatographed products by evaporation, followed by lyophilization.

While separation of multiple products can be done by HPLC, another strategy is to use nucleosides or nucleotides which contain only a single reactive functionality, whether because only one is present, or by the use of protecting groups to block side reactions at other positions in the molecule. This can be done at the level of preformed dinucleotide polyphosphates, or alternately, can be carried out on nucleoside mono-, di-, or triphosphates, leading to novel products in their own right, or can be coupled to other nucleoside mono-, di, or triphosphates by the methods which have already been described.

It will be recognized by those skilled in the art that the above reactions and purification techniques can also be applied to carba-ribose analogues (e.g., $D_1 = CH_2$) of nucleosides, nucleotides and their derivatives, and that the terms such as "mononucleotide" and "dinucleotide" also apply to the carba-ribose analogues and other derivatives defined by Formulae I-IV.

The inventors of the present invention have discovered compounds that are antagonists of the effect of ADP on its platelet membrane receptor, the $P2Y_{12}$ receptor. The compounds provide efficacy as antithrombotic agents by their ability to block ADP from acting at its platelet receptor site and thus prevent platelet aggregation. Thus, these compounds can provide a more efficacious antithrombotic effect than aspirin, but with less profound effects on bleeding than antagonists of the fibrinogen receptor. Since ADP-induced platelet aggregation is mediated by the simultaneous activation of both $P2Y_{12}$ and $P2Y_1$ receptors, the combined administration of the compounds described here with antagonists of platelet $P2Y_1$ receptors could potentially provide a more efficacious antithrombotic effect at concentrations of each antagonist that are below the effective concentrations to block each receptor subtype in other systems, resulting in a decrease of the potential manifestation of adverse effects. In addition, these compounds can be used in conjunction with lower doses of other platelet aggregation inhibitors which work by different mechanisms, to reduce the possible side effects of said agents. Finally, if the compounds of the present invention have sufficient binding affinity and bear a fluorescent moiety, they can find uses as biochemical probes for the $P2Y_{12}$ receptor.

The compounds of the present invention fall under the definition of general Formula I , which is further divided into general Formulae Ia (dinucleotides), Ib (mononucleotides) and Ib' (mononucleotide monophosphates). While potent and selective $P2Y_{12}$ antagonists can be found within either of these subdivisions, mononucleotides have an advantage over dinucleotides in terms of ease of synthesis and cost. In general, diphosphates and triphosphates falling under general Formula Ib are more potent antagonists at $P2Y_{12}$ than the corresponding monophosphates of Formula Ib'. However, nucleoside 5'-monophosphates and their analogues are easier to prepare and have greater chemical and biological stability. Thus, for synthetic reasons, a nucleoside 5'-monophosphate with appropriate druglike properties is sometimes more advantageous than other mononucleotides bearing more than one phosphate, or related dinucleotides.

Two preferred modifications falling under the definition of general Formula Ib' can be made to nucleoside 5'-monophosphates to render them antagonists of the platelet $P2Y_{12}$ receptor. In general, the preferred nucleoside 5'- monophosphate starting material chosen for this purpose is AMP, or an adenosine 5'-monophosphate derivative, as it contains the appropriate functional groups for the desired modifications and gives rise to more potent and selective antagonists compared to similar modifications of other commonly available nucleotide monophosphates. The first preferred modification is to install an aryl or aralkyl acetal bridging the 2'- and 3'- hydroxyls of the ribose, with the nature of the aryl or aralkyl group as previously described. The second modification is to add an aminocarbonyl or substituted aminocarbonyl group to the 6-amino position of the adenine base, resulting in a urea moiety at that position. Substituents on the urea moiety fall under the definition of R_{17}, as previously described. These urea substituents can be broadly categorized as either aromatic or aliphatic in nature. Generally, the most preferred substituent chosen from aryl groups is phenyl. When the urea group is an aliphatic urea, the preferred substituents on nitrogen are linear, branched, or cyclic, having from 1 to 6 carbons on the alkyl substituent, and with or without unsaturation. More preferred are linear alkyl ureas from 2 to 6 carbons inclusively, or cyclic alkyl ureas having 3 to 6 carbons in the ring; most preferred are linear alkyl ureas containing from 2 to 4 carbons inclusively in the chain or cycloalkyl ureas having from 3 to 5 carbons inclusively in the ring.

An important aspect of the present invention is that, while any of the described modifications alone can result in a compound capable of antagonism of ADP-induced platelet aggregation, it is the combination of both 2'/3' and 6-N modifications in the same molecule that renders the nucleotide a highly potent and selective $P2Y_{12}$ antagonist.

Another important aspect of the present invention is the effect of compound structure on the resultant potency in washed platelets versus potency in whole blood. In general, the potency of a given compound is lower in whole blood versus washed platelets, ostensibly the result of increased binding of the compound to the higher levels of blood proteins in the former. This property is particularly acute for nucleoside 5'-monophosphates versus the corresponding di- and triphosphates, since there are fewer ionizable groups available in the former to offset the lipophilic acetal and urea groups which, presumably, increase protein binding in whole blood. Unexpectedly, we found that compounds containing phenyl ureas exhibited a greater loss of potency in whole blood compared to their activity in washed platelets, while those derivatives having aliphatic ureas gave more comparable results when

tested in whole blood and washed platelet assays. Furthermore, compounds with aliphatic ureas were significantly more potent than their aromatic counterparts in washed platelets. Taken together, these findings enabled the discovery of the most potent and selective compounds of the present invention.

The compounds of general Formula I are useful in therapy, in particular in the prevention of platelet aggregation. The compounds of the present invention are thus useful as anti-thrombotic agents, and are thus useful in the treatment or prevention of unstable angina, coronary angioplasty (PTCA) and myocardial infarction.

The compounds of the present invention are also useful in the treatment or prevention of primary arterial thrombotic complications of atherosclerosis such as thrombotic stroke, peripheral vascular disease, myocardial infarction without thrombolysis.

Still further indications where the compounds of the invention are useful are for the treatment or prevention of arterial thrombotic complications due to interventions in atherosclerotic disease such as angioplasty, endarterectomy, stent placement, coronary and other vascular graft surgery.

Still further indications where the compounds of the invention are useful are for the treatment or prevention of thrombotic complications of surgical or mechanical damage such as tissue salvage following surgical or accidental trauma, reconstructive surgery including skin flaps, and "reductive" surgery such as breast reduction.

The compounds of the present invention are also useful for the prevention of mechanically-induced platelet activation in vivo such as cardiopulmonary bypass (prevention of microthromboembolism), prevention of mechanically-induced platelet activation in vitro such as the use of the compounds in the preservation of blood products, e.g. platelet concentrates, prevention of shunt occlusion such as renal dialysis and plasmapheresis, thrombosis secondary to vascular damage/inflammation such as vasculitis, arteritis, glomerulonephritis and organ graft rejection.

Still further indications where the compounds of the present invention are useful are indications with a diffuse thrombotic/platelet consumption component such as disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, heparin-induced thrombocytopenia and pre-eclampsia/eclampsia.

Still further indications where the compounds of the invention are useful are for the treatment or prevention of venous thrombosis such as deep vein thrombosis, veno-occlusive disease, hematological conditions such as thrombocythemia and polycythemia, and migraine.

In a particularly preferred embodiment of the present invention, the compounds are used in the treatment of unstable angina, coronary angioplasty and myocardial infarction.

In another particularly preferred embodiment of the present invention, the compounds are useful as adjunctive therapy in the prevention of coronary arterial thrombosis during the management of unstable angina, coronary angioplasty and acute myocardial infarction, i.e. perithrombolysis. Agents commonly used for adjunctive therapy in the treatment of thrombotic disorders can be used, for example heparin and/or aspirin, just to mention a few.

A method of treating a mammal to alleviate the pathological effects of atherosclerosis and arteriosclerosis, acute MI, chronic stable angina, unstable angina, transient ischemic attacks and strokes, peripheral vascular disease, arterial thrombosis, preeclampsia, embolism, restenosis or abrupt closure following angioplasty, carotid endarterectomy, and anastomosis of vascular grafts.

The compounds of this invention can be used in vitro to inhibit the aggregation of platelets in blood and blood products, e.g. for storage, or for ex vivo manipulations such as in diagnostic or research use. This invention also provides a method of inhibiting platelet aggregation and clot formation in a mammal, especially a human, which comprises the internal administration of a compound of Formula (I) and a pharmaceutically acceptable carrier.

Chronic or acute states of hyper-aggregability, such as disseminated intravascular coagulation (DIC), septicemia, surgical or infectious shock, post-operative and post-partum trauma, cardiopulmonary bypass surgery, incompatible blood transfusion, abruptio placenta, thrombotic thrombocytopenic purpura (TTP), snake venom and immune diseases, are likely to be responsive to such treatment.

This invention further provides a method for inhibiting the reocclusion of an artery or vein following fibrinolytic therapy, which comprises internal administration of a compound of Formula (I) and a fibrinolytic agent. When used in the context of this invention, the term fibrinolytic agent is intended to mean any compound, whether a natural or synthetic product, which directly or indirectly causes the lysis of a fibrin clot. Plasminogen activators are a well known group of fibrinolytic agents. Useful plasminogen activators include, for example, anistreplase, urokinase (UK), pro-urokinase (pUK), streptokinase (SK), tissue plasminogen activator (tPA) and mutants, or variants thereof, which retain plasminogen activator activity, such as variants which have been chemically modified or in which one or more amino acids have been added, deleted or substituted or in which one or more functional domains have

been added, deleted or altered such as by combining the active site of one plasminogen activator or fibrin binding domain of another plasminogen activator or fibrin binding molecule.

Extracorporeal circulation is routinely used for cardiovascular surgery in order to oxygenate blood. Platelets adhere to surfaces of the extracorporeal circuit. Platelets released from artificial surfaces show impaired hemostatic function. Compounds of the invention can be administered to prevent adhesion.

Other applications of these compounds include prevention of platelet thrombosis, thromboembolism and reocclusion during and after thrombolytic therapy and prevention of platelet thrombosis, thromboembolism and reocclusion after angioplasty of coronary and other arteries and after coronary artery bypass procedures.

The compounds of the present invention also encompass their non-toxic pharmaceutically acceptable salts, such as, but not limited to, an alkali metal salt such as sodium or potassium; an alkaline earth metal salt such as manganese, magnesium or calcium; or an ammonium or tetraalkyl ammonium salt, i.e., NX_4^+ (wherein X is C_{1-4}). Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects.

Those skilled in the art will recognize various synthetic methodologies which can be employed to prepare non-toxic pharmaceutically acceptable salts and acylated prodrugs of the compounds.

The active compounds can be administered systemically to target sites in a subject in need such that the extracellular concentration of a $P2Y_{12}$ agonist is elevated to block the binding of ADP to $P2Y_{12}$ receptor, thus inhibit the platelet aggregation. The term systemic as used herein includes subcutaneous injection, intravenous, intramuscular, intrasternal injection, intravitreal injection, infusion, inhalation, transdermal administration, oral administration, rectal administration and intra-operative instillation.

For systemic administration such as injection and infusion, the pharmaceutical formulation is prepared in a sterile medium. The active ingredient, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Adjuvants such as local anesthetics, preservatives and buffering agents can also be dissolved in the vehicle. The sterile indictable preparation can be a sterile indictable solution or suspension in a non-toxic acceptable diligent or solvent. Among the acceptable vehicles and solvents that can be employed are sterile water, saline solution, or Ringer's solution.

Another method of systemic administration of the active compound involves oral administration, in which pharmaceutical compositions containing active compounds are in the form of tablets, lozenges, aqueous or oily suspensions, viscous gels, chewable gums, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

For oral use, an aqueous suspension is prepared by addition of water to dispersible powders and granules with a dispersing or wetting agent, suspending agent one or more preservatives, and other excipients. Suspending agents include, for example, sodium carboxymethylcellulose, methylcellulose and sodium alginate. Dispersing or wetting agents include naturally-occurring phosphatides, condensation products of an allylene oxide with fatty acids, condensation products of ethylene oxide with long chain aliphatic alcohols, condensation products of ethylene oxide with partial esters from fatty acids and a hexitol, and condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anydrides. Preservatives include, for example, ethyl, and n-propyl p-hydroxybenzoate. Other excipients include sweetening agents (e.g., sucrose, saccharin), flavoring agents and coloring agents. Those skilled in the art will recognize the many specific excipients and wetting agents encompassed by the general description above.

For oral application, tablets are prepared by mixing the active compound with nontoxic pharmaceutically acceptable excipients suitable for the manufacture of tablets. These excipients can be, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example, starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate can be employed. Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin or olive oil. Formulation for oral use can also be presented as chewable gums by embedding the active ingredient in gums so that the active ingredient is slowly released upon chewing.

Additional means of systemic administration of the active compound to the target platelets of the subject would involve a suppository form of the active compound, such that a

therapeutically effective amount of the compound reaches the target sites via systemic absorption and circulation.

For rectal administration, the compositions in the form of suppositories can be prepared by mixing the active ingredient with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the compound. Such excipients include cocoa butter and polyethylene glycols.

The active compounds can also be systemically administered to the platelet aggregation sites through absorption by the skin using transdermal patches or pads. The active compounds are absorbed into the bloodstream through the skin. Plasma concentration of the active compounds can be controlled by using patches containing different concentrations of active compounds.

One systemic method involves an aerosol suspension of respirable particles comprising the active compound, which the subject inhales. The active compound would be absorbed into the bloodstream via the lungs, and subsequently contact the target platelets in a pharmaceutically effective amount. The respirable particles can be liquid or solid, with a particle size sufficiently small to pass through the mouth and larynx upon inhalation; in general, particles ranging from about 1 to 10 microns, but more preferably 1-5 microns, in size are considered respirable.

Another method of systemically administering the active compounds to the platelet aggregation sites of the subject involves administering a liquid/liquid suspension in the form of eye drops or eye wash or nasal drops of a liquid formulation, or a nasal spray of respirable particles that the subject inhales. Liquid pharmaceutical compositions of the active compound for producing a nasal spray or nasal or eye drops can be prepared by combining the active compound with a suitable vehicle, such as sterile pyrogen free water or sterile saline by techniques known to those skilled in the art.

Intravitreal delivery can include single or multiple intravitreal injections, or via an implantable intravitreal device that releases $P2Y_{12}$ antagonists in a sustained capacity. Intravitreal delivery can also include delivery during surgical manipulations as either an adjunct to the intraocular irrigation solution or applied directly to the vitreous during the surgical procedure.

For systemic administration, plasma concentrations of active compounds delivered can vary according to compounds; but are generally $1x10^{-10}$-$1x10^{-5}$ moles/liter, and preferably $1x10^{-8}$-$1x10^{-6}$ moles/liter.

The pharmaceutical utility of $P2Y_{12}$ antagonist compounds of this invention is indicated by their inhibition of ADP-induced platelet aggregation. This widely used assay, as described in S.M.O. Hourani *et al. Br. J. Pharmacol.* 105, 453-457 (1992) relies on the measurement of the aggregation of a platelet suspension upon the addition of an aggregating agent such as ADP.

The present invention also provides novel compounds. The present invention additionally provides novel pharamaceutical formulations comprising compounds of Formula I of high purity, and/or in a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier can be selected by those skilled in the art using conventional criteria. The pharmaceutically acceptable carrier include, but are not limited to, saline and aqueous electrolyte solutions, water polyethers such as polyethylene glycol, polyvinyls such as polyvinyl alcohol and povidone, cellulose derivatives such as methylcellulose and hydroxypropyl methylcellulose, petroleum derivatives such as mineral oil and white petrolatum, animal fats such as lanolin, polymers of acrylic acid such as carboxypolymethylene gel, vegetable fats such as peanut oil and polysaccharides such as dextrans, and glycosaminoglycans such as sodium hyaluronate and salts such as sodium chloride and potassium chloride.

Novel compounds of the present invention include compounds of Formula Ib (mononucleotide), provided that when n = 1, both X_1 and X_2 are not O; and when n = 0, X_1 is not O; and provided when Y' = H, that X_2 is independently O, CH_2, CHF, CHCl, CF_2, CCl_2; also provided that when $R_{10} = NH_2$ or O, and when R_5 and R_6 are taken together as oxygen doubly bonded to C, then R_7 is not equal to ortho-methylamino phenyl; further provided that when n = p = 1, $X_2 = CH_2$ and B' = adenosine, then R_1 and R_2 are not equal to napththylenylmethyl, napthylenylmethylene, or phenylmethylene.

Novel compounds of the present invention also include compounds of Formula Ia, wherein B and B' are independently pyrimidine (pyrimidine/pyrimidine dinucleotide), provided that when m + n + p = 1, $R_{16} = CH_3$, and R_5 and R_6 are taken together as oxygen doubly bonded to C, then R_7 is not equal to CH_3 (Z' does not equal to acetate); also provided that when m + n + p = 3, B and B' = uridine, and R_5 and R_6 are taken together as oxygen doubly bonded to C, then R_7 is not equal to phenyl for Y' = OR_1 and/or Y= OR_4 (Y and Y'

does not equal to benzoyl); further provided that when m + n + p = 1, then both R_8 and R_9 are not CH_3 (Z' and Y' taken together do not equal isopropylidine).

Novel compounds of the present invention also include compounds of Formula Ia, wherein B is a purine or residue according to general formula IV, and B' is a pyrimidine residue according to general formula V, (purine/pyrimidine dinucleotide); provided that Y' is not equal to OCH_3 when Z', Y, or Y' = H or OH; further provided that R_8 is not equal to OCH_2CH_3 when R_9 = H (Z' and Y' or Z and Y taken together do not equal to an orthoethylester).

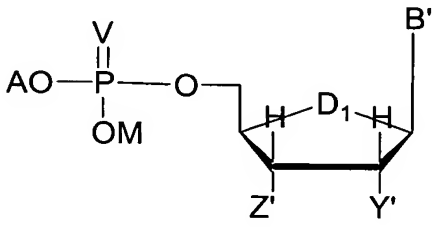
Novel compounds of the present invention also include compounds of Formula Ia, wherein B and B' are independently a purine residue according to general formula IV, (purine/purine dinonucleotide); provided that (a)Y or Y' is not equal to OCH_3 when R_{10} = NH_2 or O; (b) R_8 is not equal to OCH_3 or OCH_2CH_3 when R_9 = H; (c) both R_8 and R_9 are not equal to CH_3; (d) when m+n+p = 1, then R_8 and R_9 does not equal OCH_2CH_3; (e) when R_{10} = NH_2, and when R_5 and R_6 are taken together as oxygen doubly bonded to C, then R_7 is not equal to ortho-methylaminophenyl; (f) when m+n+p = 1, and when R_5 and R_6 are taken together as oxygen doubly bonded to C, then R_7 is not equal to $CH(CH_2CH_2SCH_3)NHS(o\text{-}NO_2\text{-}Ph)$ or $CH(CH_2Ph)NHS(o\text{-}NO_2\text{-}Ph)$.

Preferred compounds of the present invention include 2'- or 3'- phenylcarbamate UTP, 2', 3'- di-phenylcarbamate UTP, 2', 3'- phenylacetaldehyde acetal ADP, di[3'(phenylcarbamate)dUp2dU], 2', 3'- phenylacetaldehyde acetal Up3U, di 2', 3'- phenylacetaldehyde acetal Up3U, 2', 3'- phenylacetaldehyde acetal Up4A, 2', 3'- phenylacetaldehyde acetal Ap4U, di 2', 3'- phenylacetaldehyde acetal Ap4U, 2', 3'- phenylacetaldehyde acetal Ip4U, 2', 3'-phenylacetaldehyde acetal Up4U, 2', 3'- phenylacetaldehyde acetal Ip4U, 2', 3'- phenylacetaldehyde acetal Up4dC, tetraphenylcarbamate Up4U, di2', 3'- benzaldehyde acetal Ip4U, di 2', 3'- benzaldehyde acetal Up4U, 2', 3'- benzaldehyde acetal Up4U, di 2', 3'- phenylacetaldehyde acetal Cp4U, 2', 3'- phenylacetaldehyde acetal Cp4U, 2', 3'- phenylacetaldehyde acetal Up4C, 2', 3'- phenylacetaldehyde acetal Up4T, di 2', 3'- benzaldehyde acetal Cp4U, 2', 3'- benzaldehyde acetal Ip4U, 2', 3'- benzaldehyde acetal Up4U, 2', 3'- benzaldehyde acetal Up4dC, 2', 3'- benzaldehyde acetal Cp4U, 2', 3'- benzaldehyde acetal Up4C, 2', 3'- phenylpropionaldehyde acetal Up4U, di 2', 3'- phenylpropionaldehyde acetal Up4U, 2', 3'- benzaldehyde acetal Cp4C, Bis MANT Up4U, Mant Up4U, Di 2', 3'- benzylacetal Up4U, Mono 2', 3'-

benzylacetal Up4U, Triphenyl carbamate Up4U, 2', 3'- phenylcarbamate Up4U, and monophenylcarbamate Up4U.

Novel mononucleoside 5'- monophosphates compounds include compounds of Formula Ib:

Formula Ib'



wherein:

V = O;

A= M;

M = H or a pharmaceutically-acceptable inorganic or organic counterion;

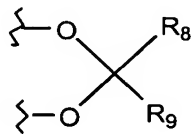
D_1 = O;

Y' =H, OH, or OR_1;

Z' =H, OH, or OR_2; with the proviso that at least one of Y' and Z' is OR_1 or OR_2;

R_1 and R_2 are residues which are linked directly to the 2' and /or 3' hydroxyls of the furanose or carbocycle via a carbon atom according to Formula II, or linked directly to two of the 2' and 3' hydroxyls of the furanose or carbocycle via a common carbon atom according to Formula III,

Formula III



wherein:

the O atoms are the 2'- and 3'- oxygens of the furanose; and

the 2'- and 3'- oxygens of the furanose are linked by a common carbon atom to form a cyclical acetal; and

R_8 is hydrogen; and

R_9 is selected from the group consisting of aralkyl, aryl, substituted aralkyl, and substituted aryl;

in which the aralkyl groups are straight chained from 1 to 5 carbons, with or without unsaturation and without heteroatoms in the alkyl portion, and are monocyclic moieties from 5 to 6 carbons in the aryl portion; and the aryl groups are monocyclic moieties from 4 to 6 carbons, with or without heteroatoms;

B' is a purine residue according to general Formula IV

wherein:

R_{10} is acylamino, according to Formula VI; and

R_{17} is amino or mono- or disubstituted amino such that the moiety according to Formula VI is a urea;

J = carbon;

R_{11} is absent;

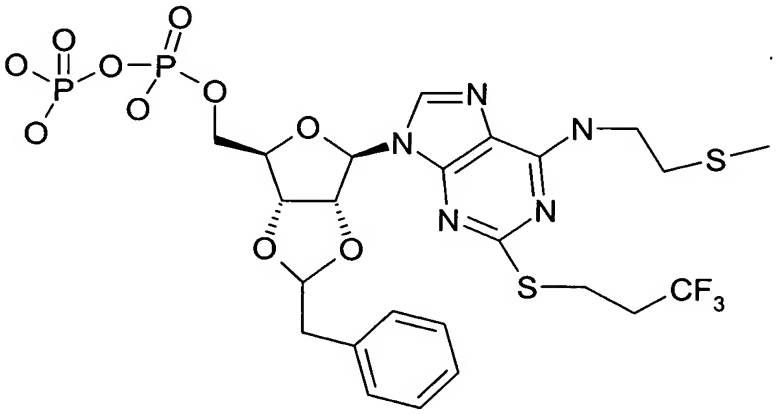
R_{12} is hydrogen; and

R_{13} is hydrogen.

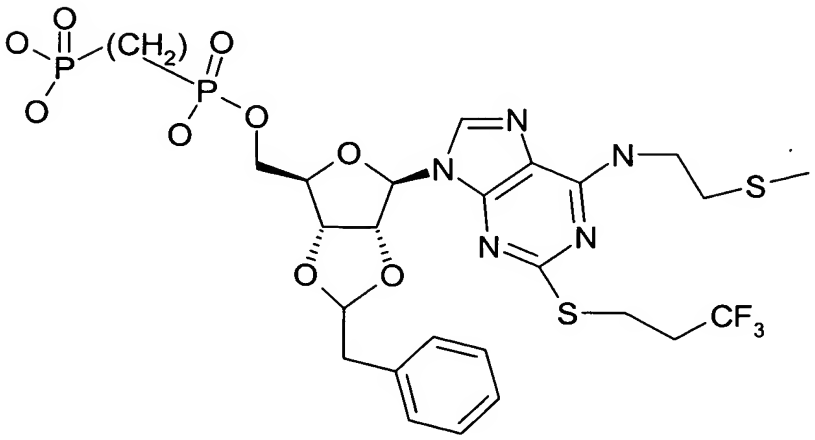
For mononucleoside 5'- monophosphates, preferred compounds of the present invention include 2', 3'-phenylacetaldehyde acetal-6-N-phenylurea AMP (compound 22), 2', 3'- phenylacetaldehyde acetal-6-N-n-hexylurea AMP (compound 23), 2', 3'-phenylacetaldehyde acetal-6-N-ethylurea AMP (compound 24), 2', 3'- phenylacetaldehyde acetal-6-N-cyclopentylurea AMP (compound 25), 2', 3'- cinnamyl acetal-6-N-n-hexylurea AMP (compound 26), 2', 3'- cinnamyl acetal-6-N-ethylurea AMP (compound 27), 2', 3'-cinnamyl acetal-6-N-phenylurea AMP (compound 28), 2', 3'- cinnamyl acetal-6-N-n-propylurea AMP (compound 29), 2', 3'- cinnamyl acetal-6-N-n-butylurea AMP (compound 30), 2', 3'- phenylpropargyl acetal-6-N-phenylurea AMP (compound 31), 2', 3'-phenylpropargyl acetal-6-N-n-hexylurea AMP (compound 32), 2', 3'- phenylpropargyl acetal-6-N-n-butylurea AMP (compound 33), 2', 3'- phenylpropargyl acetal-6-N-n-propylurea AMP (compound 34), 2', 3'-phenylpropargyl acetal-6-N-ethylurea AMP (compound 35), 2', 3'- benzaldehyde acetal-6-N-ethylurea AMP (compound 36), 2', 3'-benzaldehyde acetal-6-N-n-propylurea AMP (compound 37), 2', 3'- benzaldehyde acetal-6-

N-n-butylurea AMP (compound 38), 2', 3'- benzaldehyde acetal-6-N-n-hexylurea AMP (compound 39), and 2', 3'- benzaldehyde acetal-6-N-cyclopentylurea AMP (compound 40).

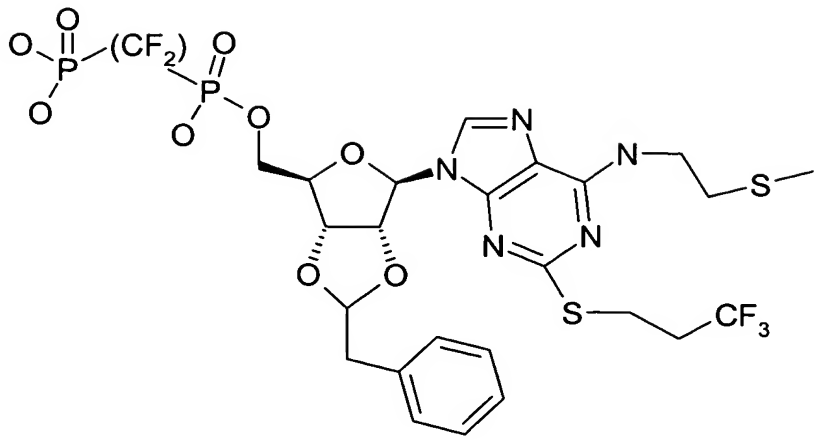
Preferred compositions also comprise the following Compounds 1-40. In the following structures hydrogens which are understood to be present have been omitted for the sake of simplicity. Tautomers drawn represent all tautomers possible. As diastereomers are generated with the introduction of the acetal group, structures containing this moiety are taken to mean either of the possible diastereomers alone or a mixture of diasteromers in any ratio.



Compound 1

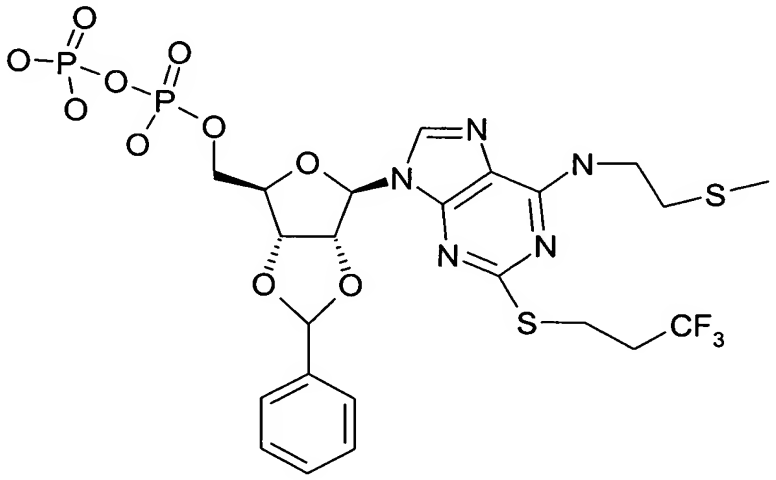


Compound 2



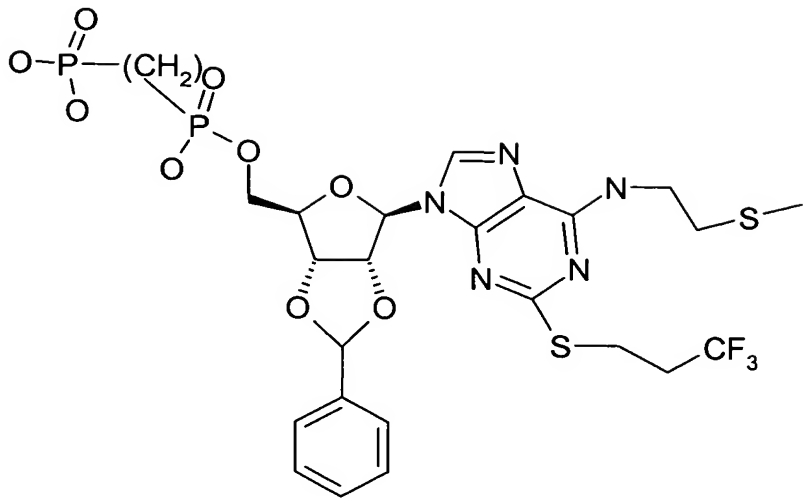
Compound 3

2-(3-trifluoromethylpropyl)thio-6-(2-methylthio) ethylamino-2',3'-(benzyl)methylenedioxy purine riboside 5'-α,β-difluoromethylene diphosphate

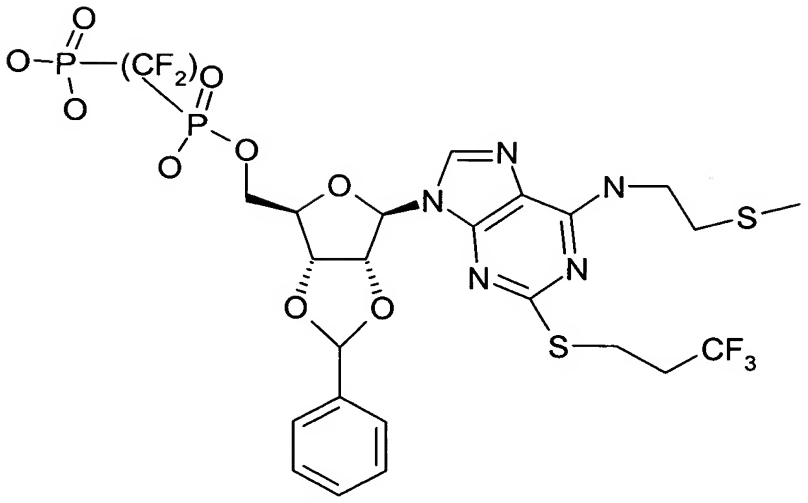


Compound 4

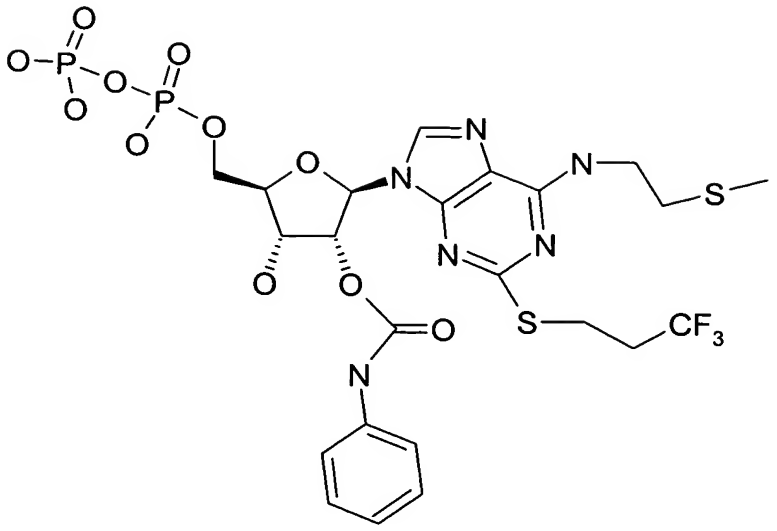
5



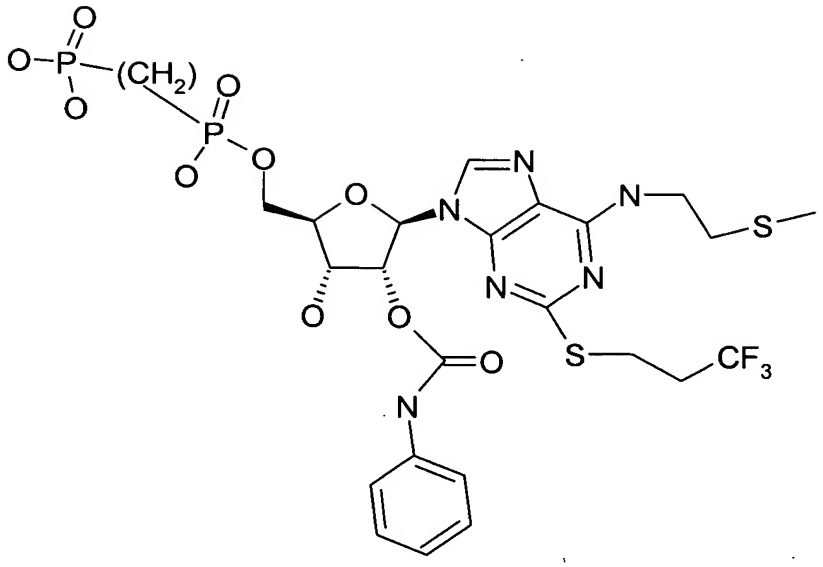
Compound 5



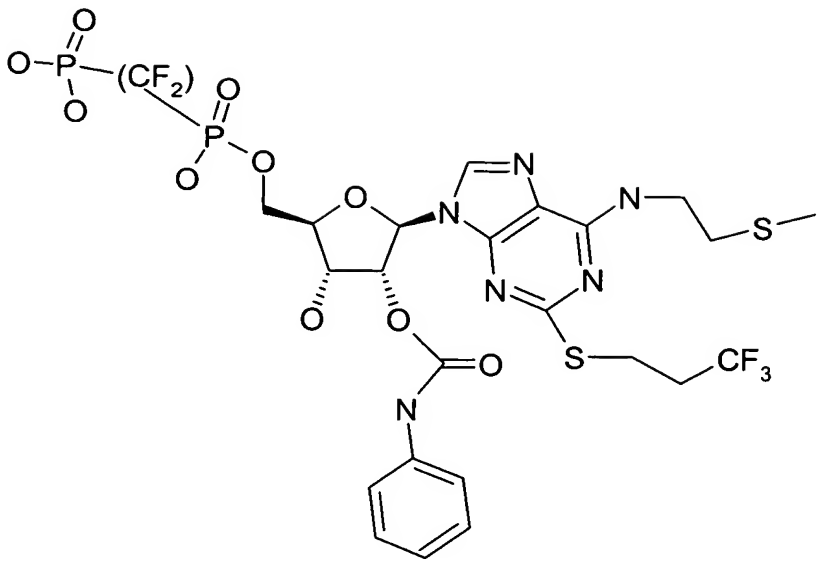
Compound 6



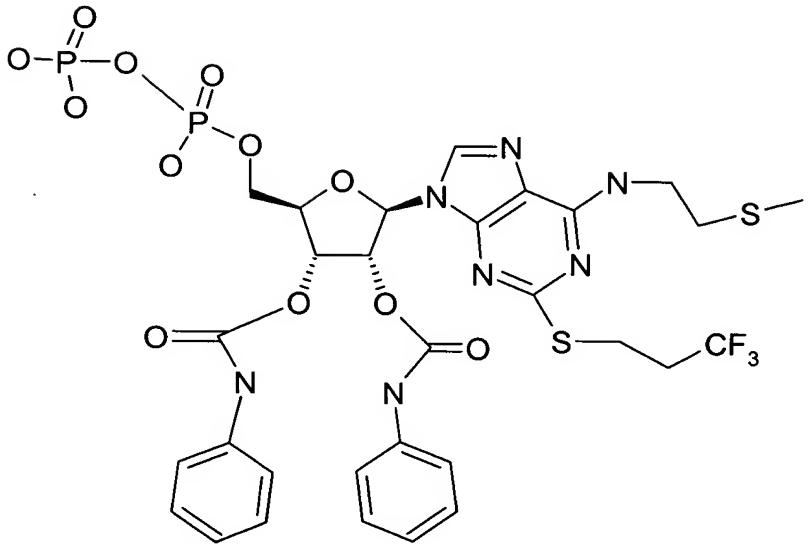
Compound 7



Compound 8



Compound 9



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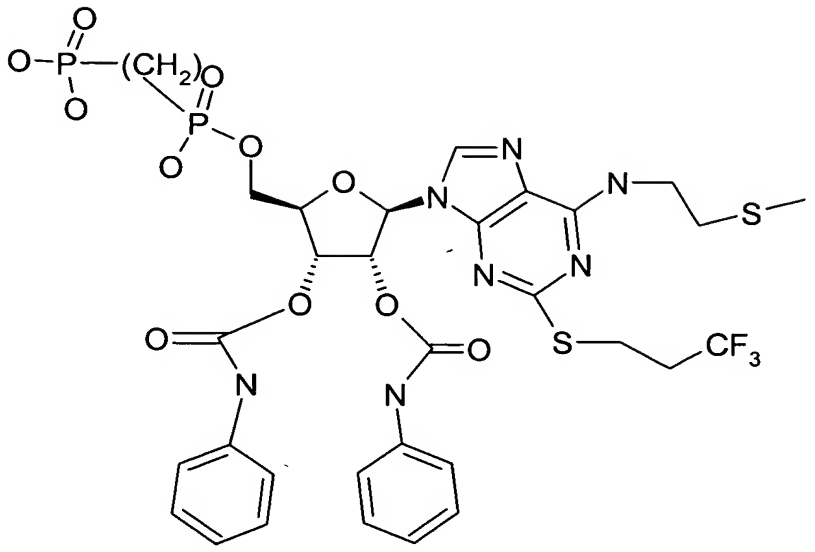
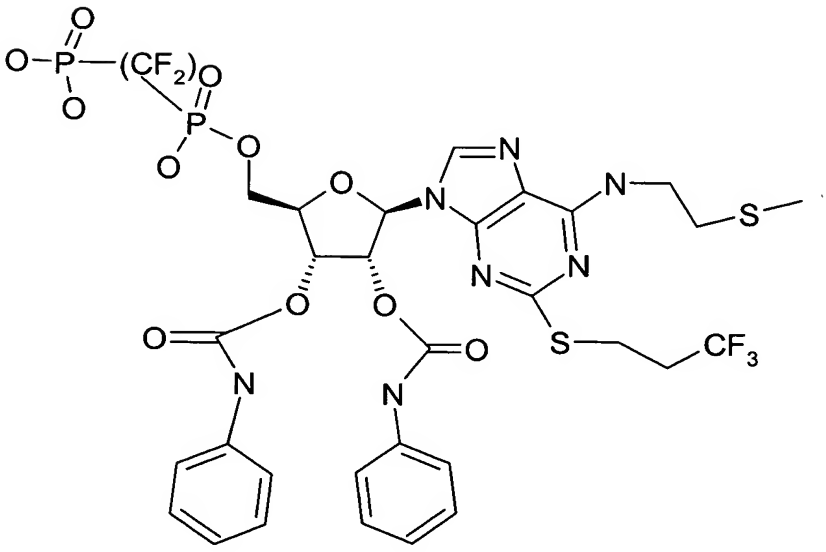


Compound 11



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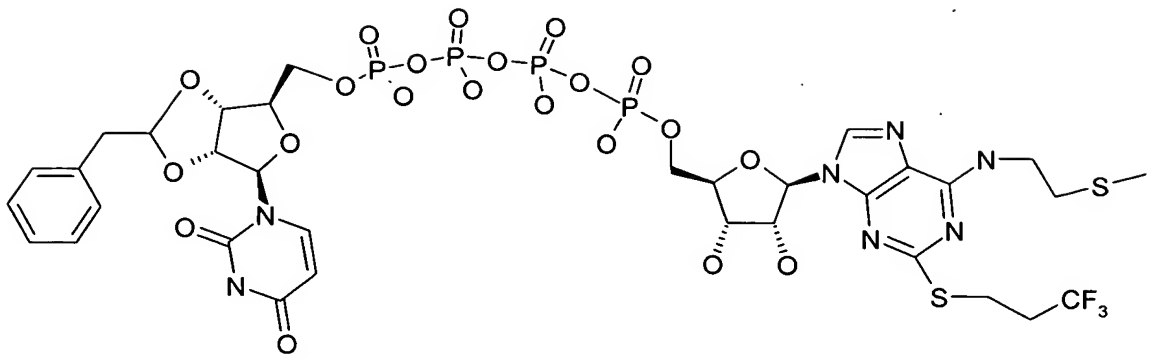
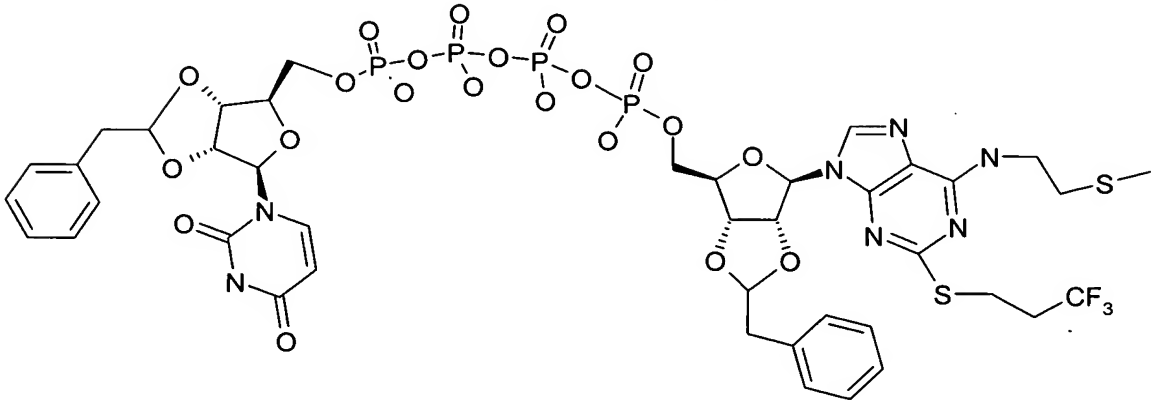
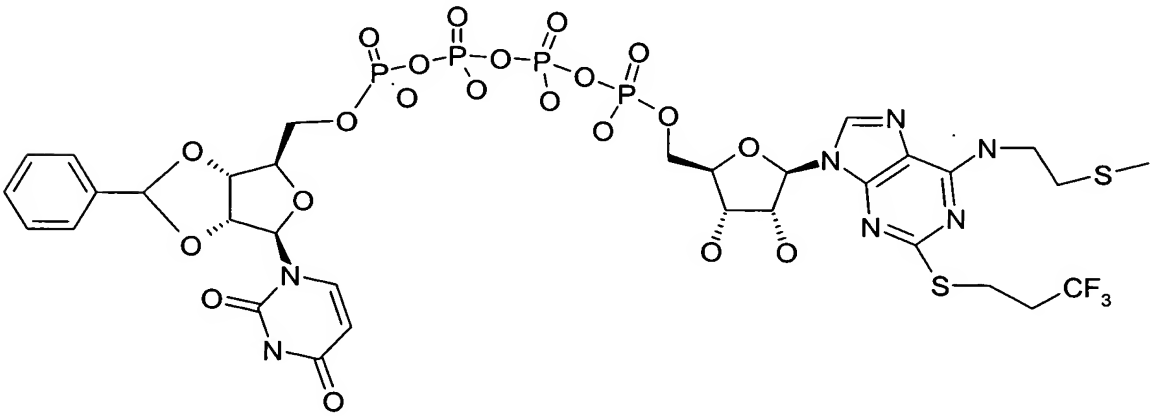


Compound 13

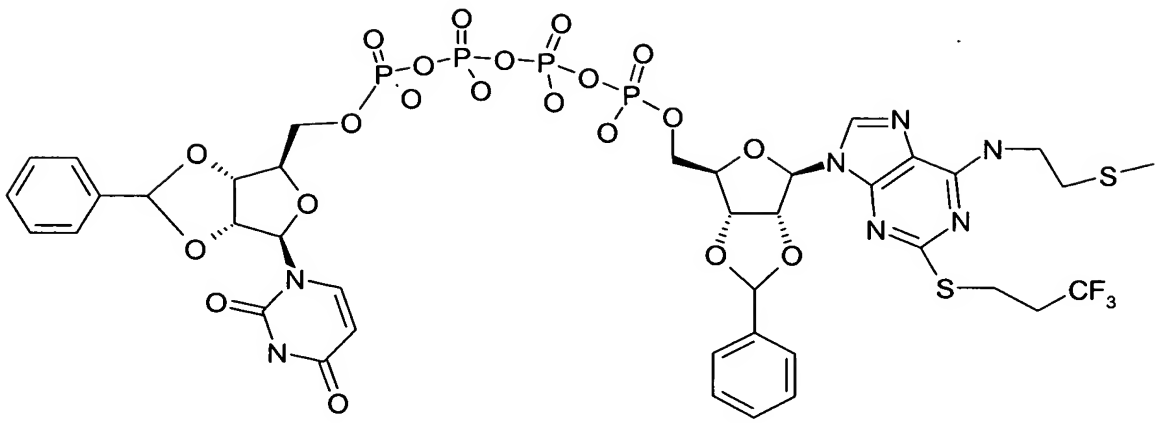


Compound 14

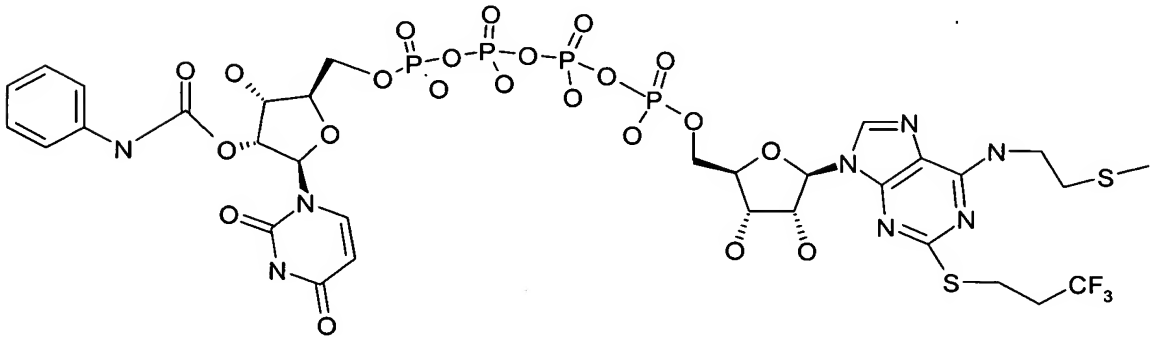
P^1-[2-(3-trifluoromethylpropyl)thio-6-(2-methylthio)ethylamino 2',3'-(benzyl)methylene dioxy purine riboside]-P^4-(2',3'-(benzyl)methylene dioxy uridine) tetraphosphate



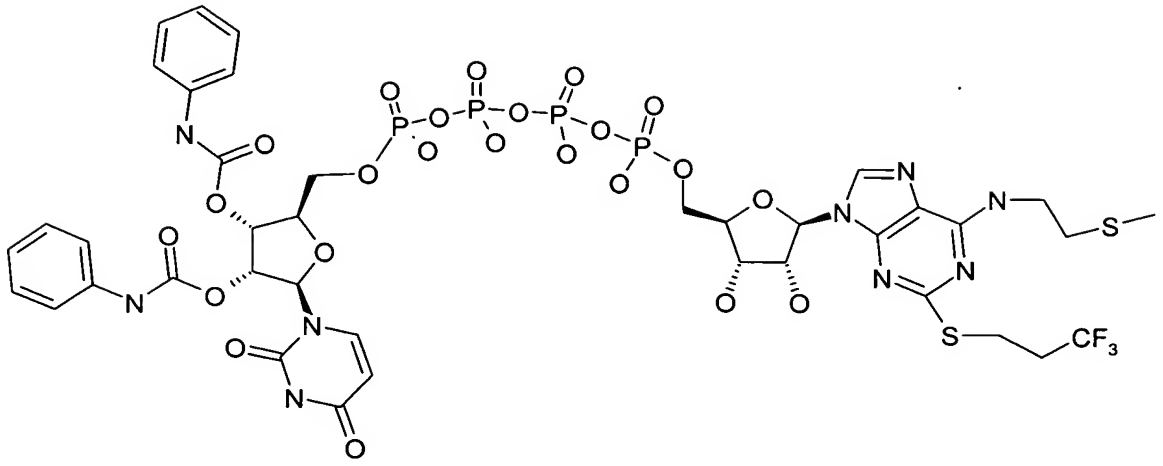
Compound 15



Compound 16



Compound 17



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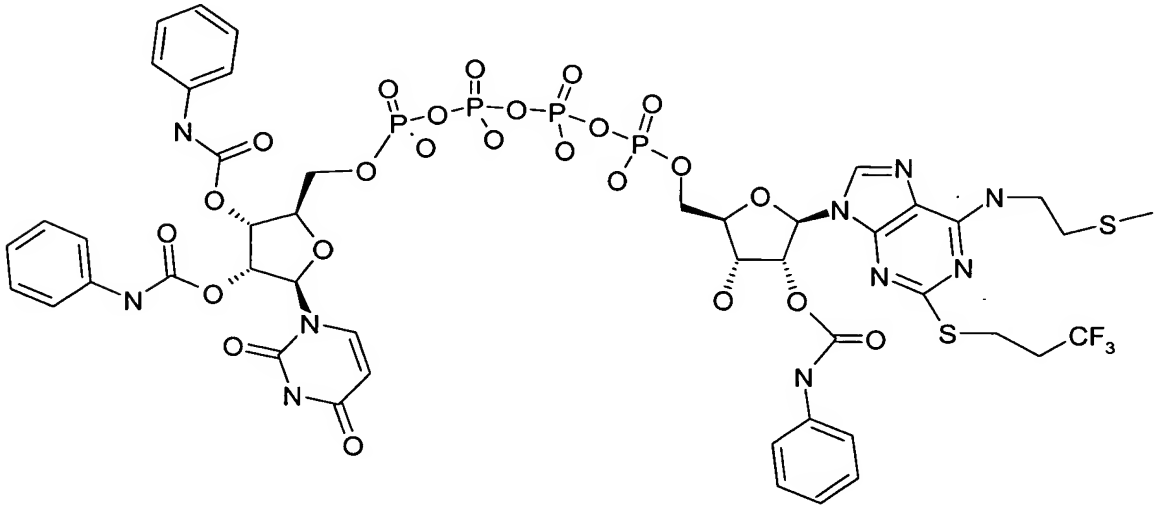

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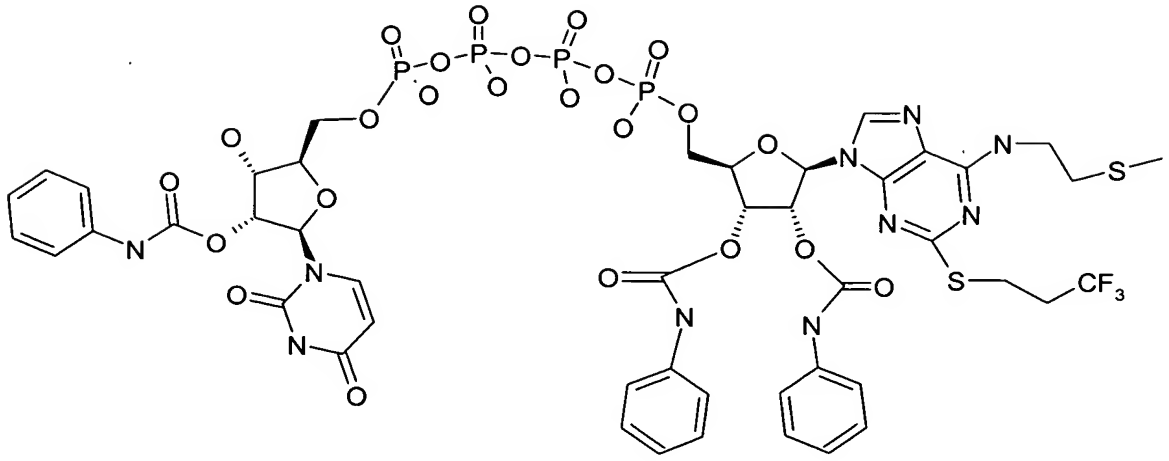
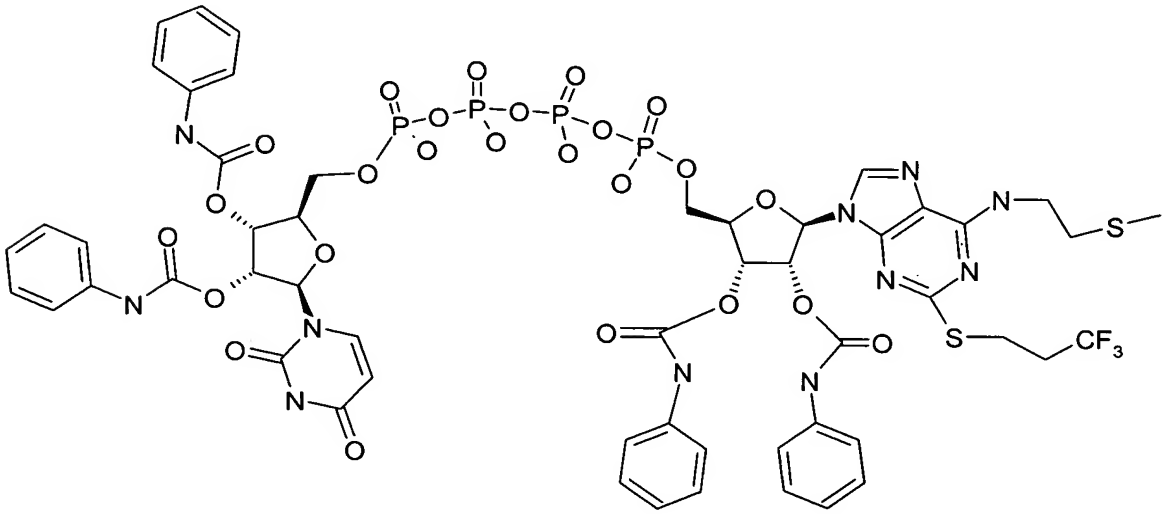


Compound 20



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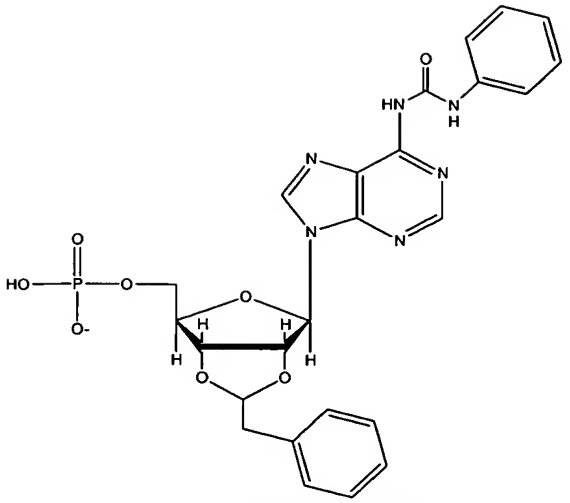
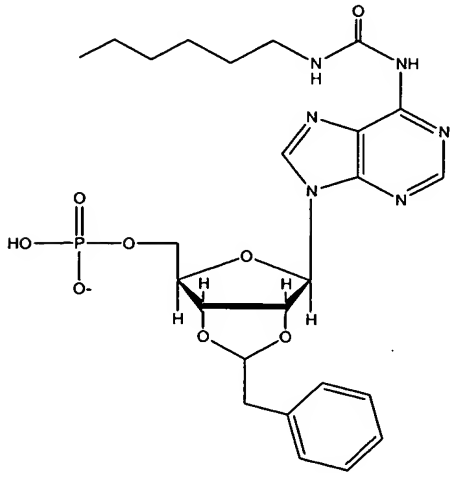


Compound 22



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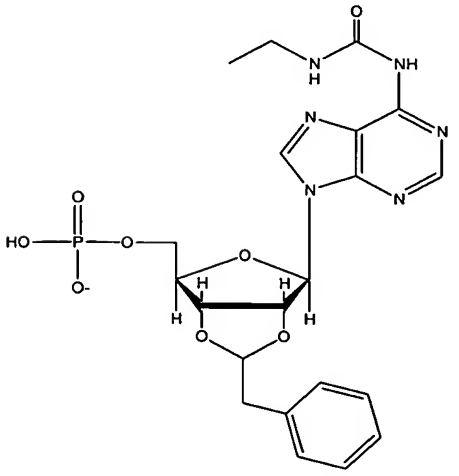

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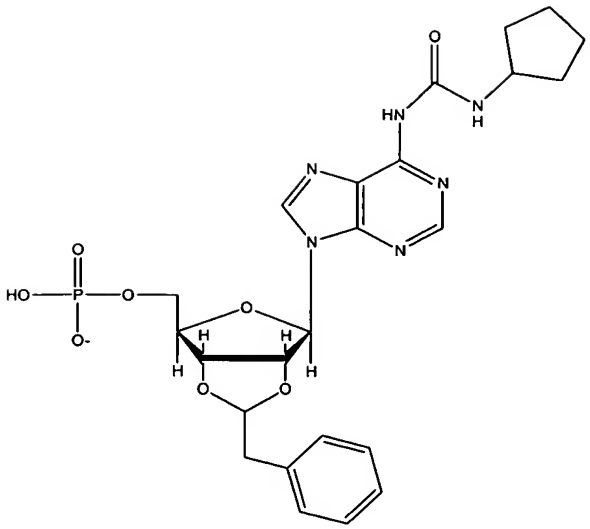

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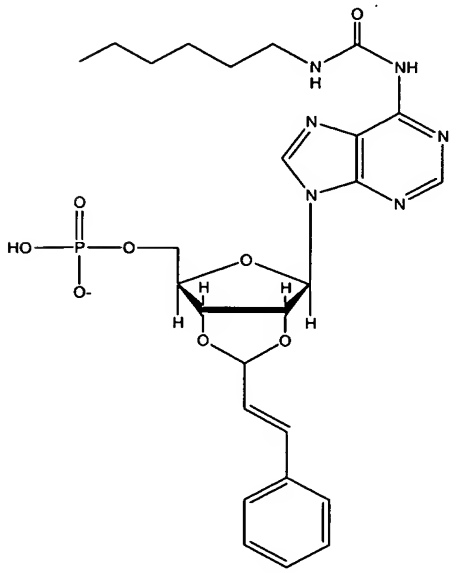

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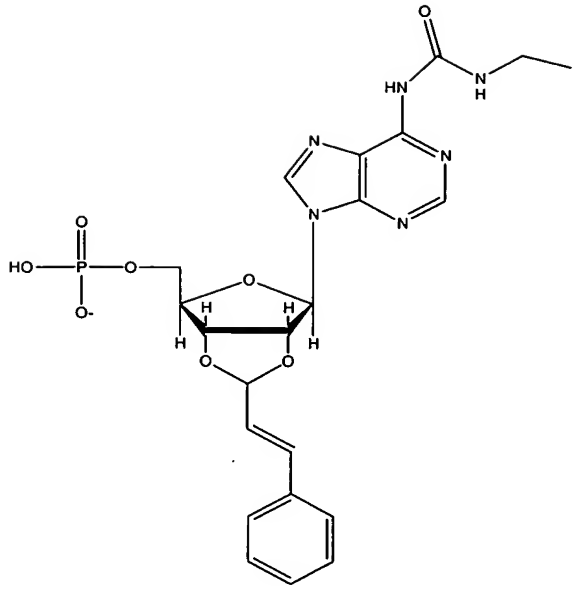

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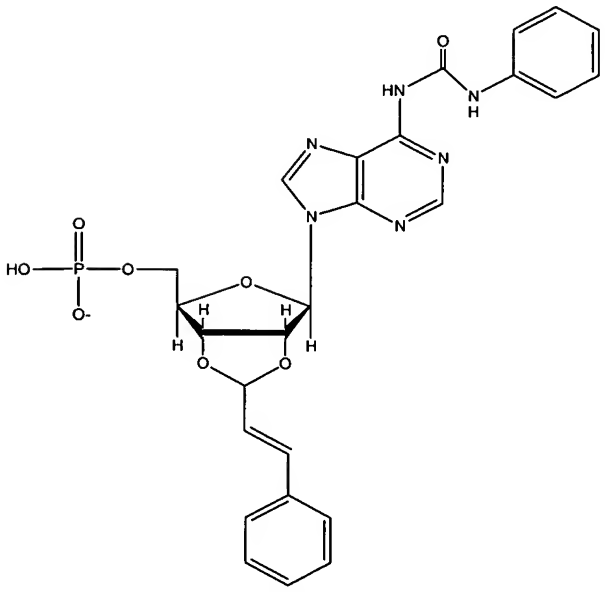

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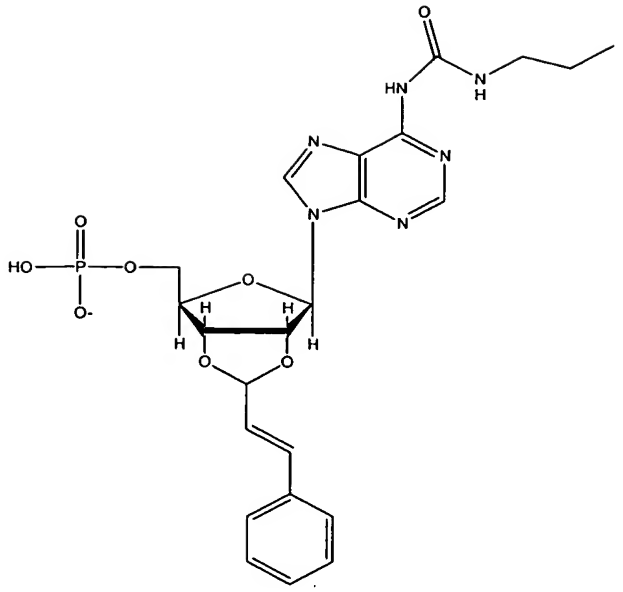

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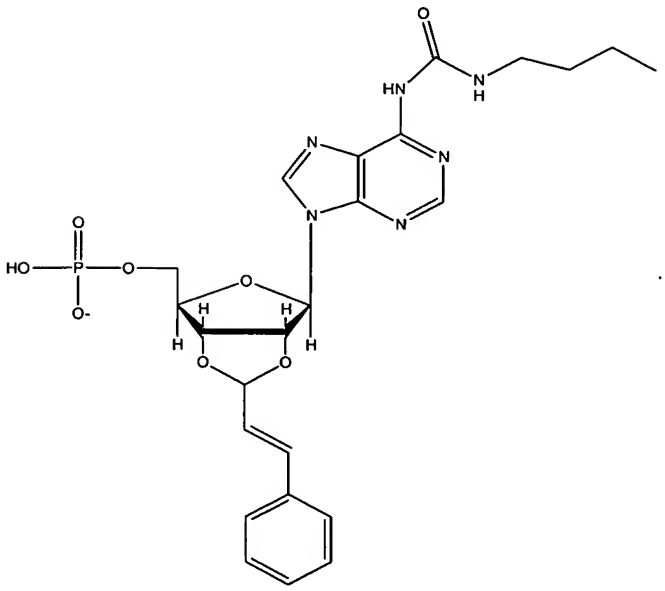
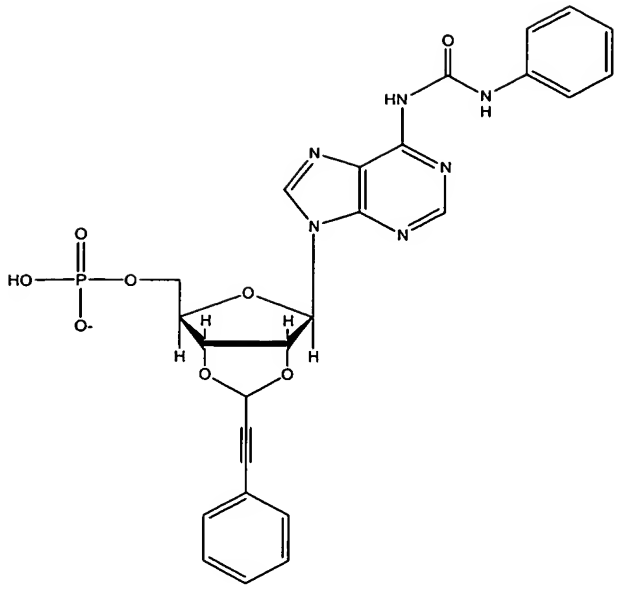


Compound 30



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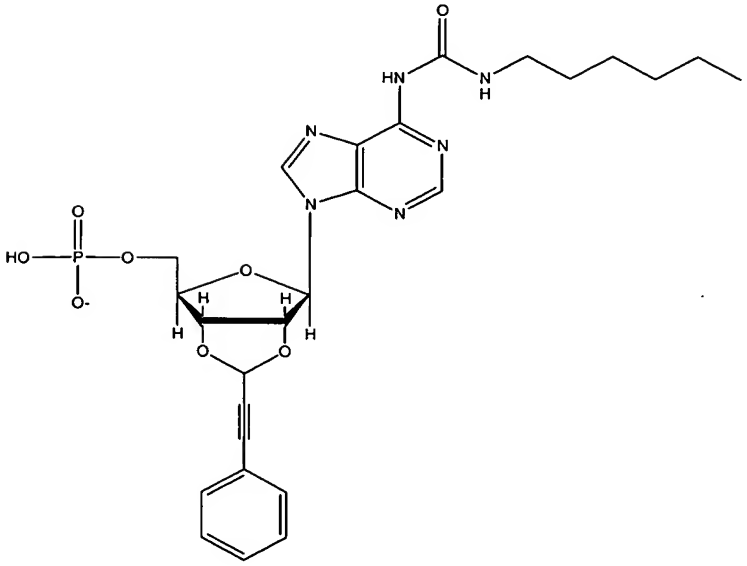

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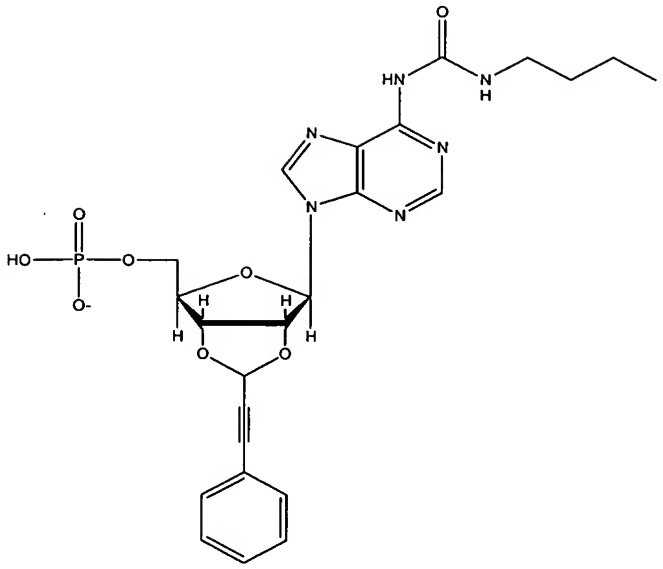

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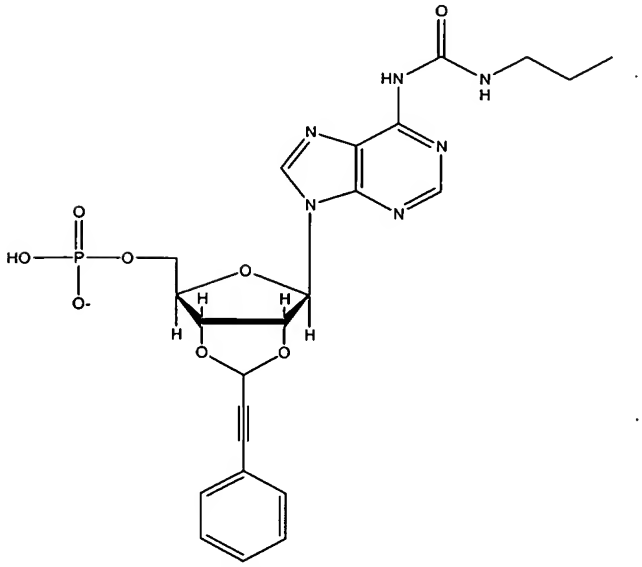

Compound 34

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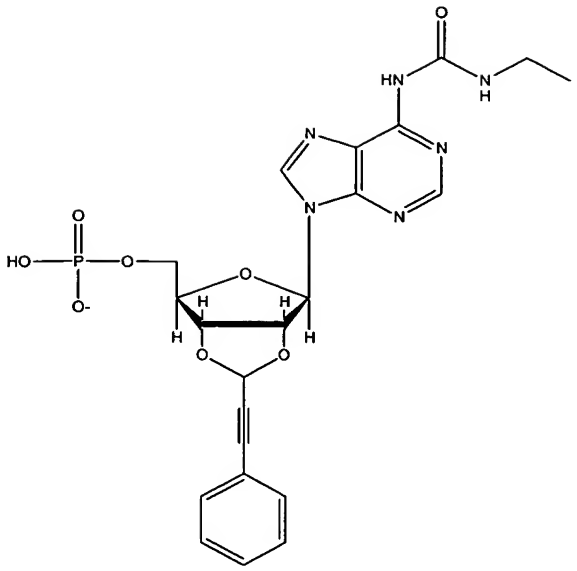

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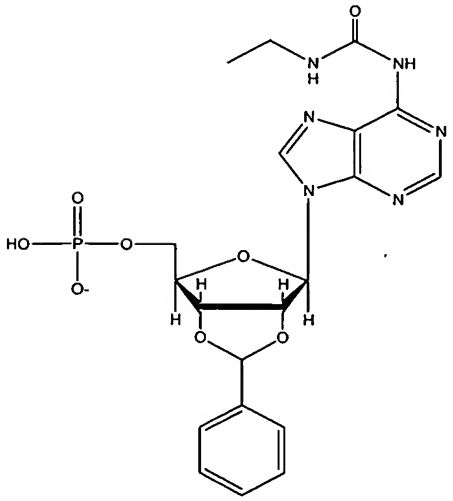

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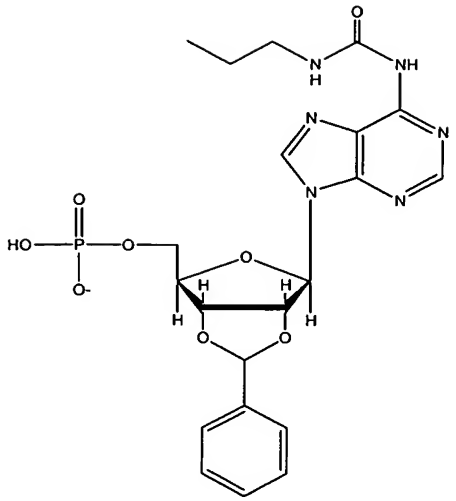

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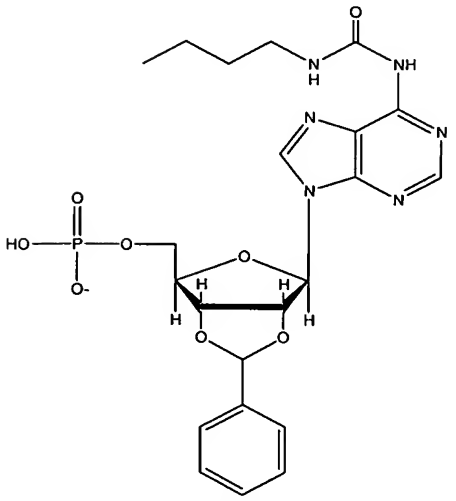
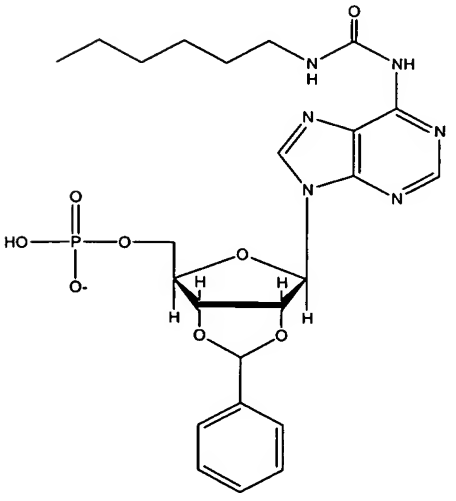
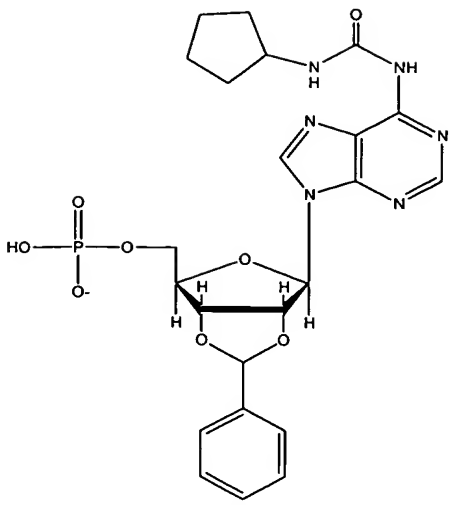


Compound 38



Compound 39



Compound 40

The invention is illustrated further by the following examples that are not to be construed as limiting the invention in scope to the specific procedures described in them.

EXAMPLES

Example 1

2'(3')-O-((phenylaminocarbonyl) -uridine 5'-)triphosphate

Uridine 5'- triphosphate, ditributylammonium salt (100 mg, 0.176 mmol; prepared from the trisodium salt by treatment with Dowex 50Wx4 H^+ in water, followed by mixing the protonated species with an excess of tributylamine, stripping and lyophilization) was dissolved in dry DMF (1 mL) and phenylisocyanate (19 μL, 0.176 mmol) added. The reaction mixture was heated at 45°C for 15 minutes, at which point a further portion of phenylisocyanate (19 μL, 0.176 mmol) was added. The solution was heated at 45°C overnight and the DMF was removed on a rotary evaporator. The residual oil was partitioned between water (2 mL) and ethyl acetate (2 mL) and the layers were separated. The aqueous layer was extracted twice more with ethyl acetate (2 mL each) and the water was removed on a rotary evaporator. The residue was dissolved in water (1.5 mL) and the product isolated by repeated injections onto a preparative HPLC column (Alltech Nucleotide/Nucleoside C18, 7um, 10 X 250 mm, gradient from 0.1 M ammonium acetate to methanol over 30 minutes, 5

mL/min, monitor at 260 nm). The yield of the carbamate was 26mg (22%, calculated for the tetraammonium salt). 1H NMR showed the product to be a mixture of 2' and 3' carbamates. The product so obtained can be used for the purposes of this invention *per se* or can be activated with a suitable coupling agent (e.g. a carbodiimide) and reacted with a variety of nucleotides to generate novel dinucleoside polyphosphates.

1H NMR (D2O, 300 MHz): δ 4.10-4.47 (m, 4H), 5.17 (m, 1H), 5.83 (dd, 1H), 5.96 (m, 1H), 7.04 (t, 1H), 7.25 (m, 4H), 7.79 (m, 1H). 31P NMR (D2O,121.47 MHz): δ -9.54 (m, 1P), -10.20 (m, 1P), -21.87 (m, 1P).

Example 2

2'(3')-O-(phenylaminocarbonyl) -P^1,P^4-di(uridine 5'-)tetraphosphate ["monophenylcarbamate Up4U"], Di- 2'(3')-O-(phenylaminocarbonyl) -P^1,P^4-di(uridine 5'-)tetraphosphate ["diphenylcarbamate Up4U"] and Tri- 2'(3')-O-(phenylaminocarbonyl) -P^1,P^4-di(uridine 5'-)tetraphosphate ["triphenylcarbamate Up4U"]

P^1,P^4-Di(uridine 5'-) tetraphosphate, ditributylammonium salt (211 mg, 0.182 mmol; prepared from the tetrasodium salt by treatment with Dowex 50Wx4 H^+ in water, followed by mixing the protonated species with an excess of tributylamine, stripping and lyophilization) was dissolved in dry DMF (2 mL) and phenylisocyanate (40 μL, 3.64 mmol) added in a single portion. The homogeneous reaction mixture was heated overnight at 45°C, whereupon TLC (silica gel, 50% isopropanol / 50% ammonium hydroxide) indicated a substantial conversion to two products. The solvent was removed on a rotary evaporator and the residue was partitioned between water (7 mL) and ethyl acetate (10 mL). The layers were separated, and the aqueous was extracted twice more with ethyl acetate. (10 mL each). The water was removed from the aqueous extract and the residual oil lyophilized overnight. The solid obtained was reconstituted in water (3 mL) and the two products separated by repeated injections onto a semipreparative HPLC column (Alltech Nucleotide/Nucleoside C18, 7um, 10 X 250 mm, gradient from 0.1 M ammonium acetate to methanol over 30 minutes, 5 mL/min, monitor at 260 nm). Stripping and lyophilization gave the mono-phenylcarbamate (48 mg, 27 % yield), di-phenylcarbamate (16 mg, 8%yield) and a trace amount of the triphenylcarbamate, as the tetraammonium salts. All three products were mixtures of the corresponding 2'/3' regiosiomers.

Monophenylcarbamate: ^{1}H NMR (D_2O, 300 MHz): δ 4.08-4.65 (m, 9H), 5.14 (d, 1H), 5.75-5.94 (m, 4H), 7.01 (t, 1H), 7.22 (m, 4H), 7.76 (m, 2H). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.17 (m, 2P), -21.81 (m, 2P).

Diphenylcarbamate: ^{1}H NMR (D_2O, 300 MHz): δ 4.13-4.43 (m, 8H), 5.12 (m, 2H), 5.84 (m, 4H), 7.01 (m, 2H), 7.21 (m, 8H), 7.75 (dd, 2H). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.19 (m, 2P), -21.65 (m, 2P).

Triphenylcarbamate: ^{1}H NMR (D_2O, 300 MHz): δ 4.29 (m, 7H), 4.5.10 (m, 1H), 5.27 (m, 2H), 5.87 (m, 4H), 7.09 (m, 15H), 7.76 (d, 2H). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.30 (m, 2P), -21.73 (m, 2P).

Example 3

P^1,P^4-Tetra-(2'(3')-O-(phenylaminocarbonyl) di(uridine 5'-)tetraphosphate [tetraphenylcarbamate Up4U"]

This derivative was prepared according to the method of example 2. P^1,P^4-Di(uridine 5'-) tetraphosphate, ditributylammonium salt (200 mg, 0.172 mmol) was treated with 16 eq of phenylisocyanate (300 uL, 2.76 mmol) in DMF and stirred overnight at 35°C. The solvent was evaporated and the excess reagents removed by extraction of an aqueous solution of the product with ethyl acetate. Following preparative HPLC as previously described, 93 mg (30% yield) of the tetraphenylcarbamate was obtained.

Tetraphenylcarbamate^{1}H NMR (D_2O, 300 MHz): δ 7.75 (d, 2H), 7.11 (m, 16H), 6.94 (m, 4H), 5.95 (d, 2H), 5.80 (d, 2H), 5.32 (m, 2H), 5.23 (m, 2H), 4.42 (m, 2H), 4.25 (m, 2H), 4.16 (m, 2H). ^{31}P NMR (D_2O, 121.47 MHz):): δ -10.30 (m, 2P), -22.32 (m, 2P).

Example 4

2',3'-(benzyl)methylenedioxy-P^1,P^4-di(uridine 5'-)tetraphosphate ["Mono 2'/3' benzylacetal Up4U"] and P^1,P^4-Di-(2',3'-((benzyl)methylenedioxy) di(uridine 5'-)tetraphosphate ["Di 2'/3' benzylacetal Up4U"]

P^1,P^4-Di(uridine 5'-) tetraphosphate, tetrasodium salt (290 mg, 0.332 mmol) was dissolved in 98% formic acid and phenylacetaldehyde, dimethyl acetal (110 uL, 0.662 mmol) added. The reaction was stirred at ambient temperature for 3 days, at which point TLC (silica gel, 50% isopropanol / 50% ammonium hydroxide) and HPLC (C18) showed good conversion to two less polar products. The formic acid was removed on a rotary evaporator, and the residue

partitioned between 0.7 M ammonium bicarbonate (15 mL) and butyl acetate (15 mL). The layers were separated and the aqueous was washed with a further portion of butyl acetate (10 mL). The aqueous layer was stripped and the residue lyophilized overnight. The crude product was dissolved in water (5 mL) and the components separated by preparative HPLC (Waters Novapak C18, 6um, 25 X 100 mm, gradient from 0.1 M ammonium acetate to methanol over 30 minutes, 30 mL/min, monitor at 260 nm). The yield of the monoacetal was 88 mg (28%) and of the diacetal 60mg (17%), both as the tetraammonium salts.

Monoacetal: ^{1}H NMR (D_2O, 300 MHz): δ 2.99 (d, 2H), 4.01-4.32 (m, 8H), 4.77 (m, 2H), 5.33 (m, 2H), 5.74 (d, 1H), 5.81 (m, 2H), 7.21 (m, 5H), 7.64 (d, 1H), 7.79 (d, 1H). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.18 (m, 1P), -10.78 (m, 1P), -22.00 (m, 2P).

Diacetal: ^{1}H NMR (D_2O, 300 MHz): δ 2.98 (d, 4H), 3.99 (m, 4H), 4.27 (m, 2H), 5.27 (m, 2H), 5.36 (m, 2H), 5.73 (d, J= 8.1 Hz, 2H), 7.21 (m, 10H), 7.61 (d, J= 8.1 Hz, 2H). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.57 (m, 2P), -21.81 (m, 2P).

Example 5

2',3'-((benzyl)methylenedioxy) P^1,P^3- uridine 5'-)triphosphate ["2'3' phenylacetaldehyde acetal Up3U"] and P^1,P^3-Di-(2',3'-((benzyl)methylenedioxy) uridine 5'-)triphosphate ["di 2'3' phenylacetaldehyde acetal Up3U"]

P^1,P^3-Di(uridine 5'-) triphosphate, trisodium salt (100 mg, 0.129 mmol) was dissolved in 98% formic acid and phenylacetaldehyde, dimethyl acetal (64 uL, 0.386 mmol) added. After overnight stirring at room temperature, the formic acid was removed, and the residue partitioned between 1 M sodium bicarbonate and ethyl acetate. Following removal of the organic layer, the product was purified on preparative HPLC, as previously described. Following lyophilization, 40 mg (36%) of the monoacetal and 24 mg (19%) of the diacetal were obtained.

Monoacetal: ^{1}H NMR (D_2O, 300 MHz): δ 7.7s (d, 2H), 7.54 (d, 2H), 7.16 (s, 5H), 5.70 (m, 3H), 5.31 (s, 1H), 5.23 (s, 1H), 4.66 (m, 2H), 4.10 (m, 8H), 2.93 (d, 2H). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.30 (m, 1P), 10.81 (m, 1P), -21.99 (m, 1P).

Diacetal: ^{1}H NMR (D_2O, 300 MHz): δ 7.51 (d, 2H), 7.15 (m, 10H), 5.65 (d, 2H), 5.31 (d, 2H), 5.20 (t, 2H), 4.63 (m, 2H), 4.13 (m, 2H), 3.88 (m, 4H), 2.90 (d, 4H). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.75 (m, 2P), -21.97 (m, 1P).

Example 6

P^1-2',3'-((benzyl)methylenedioxy) (uridine 5'-) P^4-(deoxycytidine 5'-) tetraphosphate ["2'3' phenylacetadehyde acetal Up4dC"]

P^1- (uridine 5'-) P^4-(deoxycytidine 5'-) tetraphosphate, tetrasodium salt (100 mg, 0.16 mmol) was dissolved in 98% formic acid (1 mL), and phenylacetaldehyde, dimethyl acetal (57 uL, 0.384 mmol) added. After overnight stirring, the formic acid was removed and the residue partitioned between 1 M sodium bicarbonate and ethyl acetate. After separation of the layers, the product was purified on preparative HPLC, as previously described. Yield 40 mg (36 %). This product was amenable to subsequent modification of the deoxy cytidine base by the procedures described in examples 9-13, giving rise to lipophilic bifunctional molecules falling within the scope of this invention.

Monoacetal: ^{1}H NMR (D_2O, 300 MHz): δ 7.98 (d, 1H), 7.62 (d, 1H), 7.21 (m, 5H), 6.11 (m, 2H), 5.74 (d, 1H), 5.39 (d, 1H), 5.31 (t, 1H), 4.77 (m, 2H), 4.45 (m, 1H), 4.32 (m, 1H), 4.03 (m, 5H), 2.99 (d, 2H), 2.29 and 2.21 (M, 2H). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.15 (m, 1P), -10.68 (m, 1P), -21.98 (m, 2P).

Example 7

3'-O-(phenylaminocarbonyl) –2'-deoxy (uridine 5')- monophosphate

Deoxyuridine 5'- monophosphate, tetrabutylammonium salt (135 mg , 0.274 mmol; prepared from the disodium salt by treatment with Dowex 50Wx4 H^+, followed by stirring the resultant neutral species with excess tributylamine , stripping and lyophilization) was dissolved in dry DMF (1 mL). Phenylisocyanate (60 uL, 0.547 mmol) was added and the mixture heated overnight at 45°C, at which time TLC (silica gel, 50% isopropanol / 50% ammonium hydroxide) and HPLC (C18) indicated a substantial conversion to a less polar product. The DMF was stripped on a rotary evaporator and the oily residue partitioned between water (10 mL) and ethyl acetate (10 mL). The layers were separated and the aqueous layer was rewashed with ethyl acetate (2 X 10 mL). The water was removed and the residue was dissolved in water (2 mL). The product was isolated by repeated injections onto semipreparative HPLC (Alltech Nucleotide/Nucleoside C18, 7um, 10 X 250 mm, gradient from 0.1 M ammonium acetate to methanol over 30 minutes, 5 mL/min, monitor at 260 nm). The yield was 67 mg as the diammonium salt (53 %).

^{1}H NMR (D_2O, 300 MHz): δ 2.21 (m, 2H), 3.84 (s, 2H), 4.13 (s, 1H), 5.08 (d, 1H), 5.63 (d, 1H), 6.06 (t, 1H), 6.89 (br. t, 1H), 7.10 (m, 4H), 7.72 (d, 1H).

^{31}P NMR (D_2O, 121.47 MHz): δ -2.31 (s).

P^1-(3'-O-(phenylaminocarbonyl)-2'-deoxyuridine 5'-)P^4-(uridine 5'-)tetraphosphate

Uridine 5'-triphosphate, ditributylammonium salt (prepared from the trisodium salt by treatment with Dowex 50Wx4 H^+, followed by stirring the resultant neutral species with excess tributylamine , stripping and lyophilization) is treated with 1.5 equivalents of dicyclohexylcarbodiimide in DMF for 2 hours at room temperature. The dicyclohexylurea is filtered off, and the resultant uridine 5'- cyclical triphosphate is treated with 3'-O-(phenylaminocarbonyl) –2'-deoxy (uridine 5')- monophosphate, which is in the monotributylammonium salt form. The reaction mixture is stirred for several days at 45°C, and the solvent is removed. The products are separated by preparative HPLC, as has been previously described.

Example 8

2'(3')-(2-methylamino)benzoyl-P^1,P^4-di(uridine 5'-)tetraphosphate ("MANT Up4U") and P^1,P^4-Di-(2'(3')-(2-methylamino)benzoyl uridine 5'-)tetraphosphate ("Bis MANT Up4U")

P^1,P^4-Di(uridine 5'-) tetraphosphate, tetrasodium salt (800 mg, 0.93 mmol) was dissolved in water (5 mL) and the pH adjusted to 7.6 by the addition of solid sodium bicarbonate. N,N-dimethylformamide (DMF, 5 mL) was added, followed by N-methylisatoic anhydride (231 mg, 1.3 mmol) and the suspension was heated at 50°C for 2.5 hrs. TLC (silica gel, 50% isopropanol, 50% ammonium hydroxide) indicated that the reaction was not done by this time, so a further portion of N-methylisatoic anhydride (100 mg, 0.56 mmol) was added and the reaction heated for another hour. The DMF was removed on a rotary evaporator and the residue was dissolved in a minimum of water and applied to a DEAE Sephadex A-25 column (3 X 60 cm). The column was eluted with a stepwise gradient from water to 1 M ammonium bicarbonate and the eluent monitored with a UV detector set at 254 nm. The two products that eluted were collected separately and the solvent was removed from each and the residue lyophilized overnight. ^{1}H NMR indicated that the first product to elute was the monoacylated compound, while the latter was the diacylated derivative, and that both were mixtures with the acylation at either the 2' or 3' hydroxyls, but without two carbamates on the same sugar. The yield of the monoaminobenzoylated product was 150 mg (16%); the yield of the diaminobenzoylated compound was 91 mg (8.7%).

Monoaminobenzoylated derivative: ^{1}H NMR (D_2O, 300MHz): δ 2.70 (s, 3H), 4.09-4.55(m, 9H), 5.34 (m, 1H), 5.71 (m, 2H), 5.83 (dd, 1H), 6.01 (m, 1H), 6.57 (m, 1H), 6.65 (m, 1H), 7.25 (t, 1H), 7.72 (d, 2H), 7.81 (m, 2H). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.20 (m, 2P), -21.83 (m,2P).

Diaminobenzoylated derivative: ^{1}H NMR (D_2O, 300 MHz): δ 2.69 (s, 6H), 4.15-4.51 (m, 8H), 5.27 (m, 2H), 5.86 (m, 4H), 6.60 (m, 4H), 7.30 (m, 2H), 7.79 (m, 4H). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.16 (m, 2P), -21.76 (m, 2P).

Example 9

P^1-(4-N-(4-methoxyphenyl)aminocarbonylcytidine 5'-) -P^4-(uridine 5'-) tetraphosphate

P^1-(cytidine 5'-) -P^4-(uridine 5'-) tetraphosphate, ditributylammonium salt (50 mg, 0.043mmol; prepared from the tetraammonium salt by treatment with Dowex 50Wx4 H^+ in water, followed by mixing the protonated species with an excess of tributylamine in methanol, stripping and lyophilization) was dissolved in dry DMF (1mL) and tributylamine (10 uL, 0.43 mmol), and p-methoxyphenylisocyanate (8.4 uL, 0.648 mmol) were added in a single portion. The homogeneous reaction mixture was heated overnight at 35°C, whereupon TLC (silica gel, 50% isopropanol / 50% ammonium hydroxide) and HPLC (C18) indicated a substantial conversion to a single product. The solvent was removed on a rotary evaporator and the residue dissolved in water (1mL). The product was isolated by repeated injections onto a semi-preparative HPLC column (Alltech Nucleotide/Nucleoside C18, 7um, 10 X 250 mm, gradient from 0.1 M ammonium acetate to methanol over 30 minutes, 5 mL/min, monitor at 260 nm). Stripping and lyophilization gave the p-methoxyphenylurea (24 mg, 55 % yield), as the tetraammonium salt.

The product so obtained can be derivatized on the 2' and/or 3' hydroxyl groups according to the foregoing methods (e.g. Examples 2-6).

^{1}H NMR (D_2O, 300 MHz): δ 3.59 (s, 3H), 4.01-4.20 (m, 10H), 5.68 (m, 3H), 6.19 (d, 1H), 6.71 (d, 2H), 7.18 (d, 2H), 7.67 (d, 1H), 8.06 (d, 1H). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.13 (m, 2P), -21.76 (m, 2P).

Example 10

P^1-((4-bromophenyl)ethenocytidine 5'-) -P^4-(uridine 5'-) tetraphosphate

P^1-(cytidine 5'-) -P^4-(uridine 5'-) tetraphosphate, tetrasodium salt (500 mg, 0.57 mmol) was dissolved in water (5 mL) and a solution of 2,4'-dibromoacetophenone (792 mg, 2.85 mmol) in DMF (15 mL) added. The mixture was heated overnight at 40°C, and a further portion of the dibromoketone (400 mg, 1.44 mmol) in DMF (5 mL) added. The rection was heated a further 5 hrs, and the solvents removed by evaporation. The residue was partitioned between water (20 mL) and ethyl acetate (25 mL) and the layers separated. The aqueous layer was washed with further ethyl acetate (2x15 mL) and the aqueous evaporated to dryness. The residue was dissolved in water (5 mL) and the product was isolated by repeated injections onto a semi-preparative HPLC column (see example 6 for conditions). The yield of the pure etheno compound was 80 mg (13.5%)

^{1}H NMR (D_2O, 300 MHz): δ 4.06 (m, 8H), 4.36 (m, 2H), 5.64 (dd, 2H), 6.07 (d, 1H), 6.74 (d, 1H), 7.45 (d, 2H), 7.54 (d, 2H), 7.59 (d, 1H), 7.63 (d, 1H), 7.93 (s, 1H). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.09 (m, 2P), -21.59 (m, 2P).

Example 11

P^1-((4-bromophenyl)etheno-2'-deoxycytidine 5'-) -P^4-(uridine 5'-) tetraphosphate

Example 11 product was prepared from 100 mg P^1-(2'-deoxycytidine 5'-) -P^4-(uridine 5'-) tetraphosphate, tetrasodium salt and 2,4'-dibromoacetophenone, according to the general method of example 10. Yield= 35 mg (30%).

^{1}H NMR (D_2O, 300 MHz): δ 2.31 (m, 2H), 4.03 (m, 8H), 5.60 (dd, 2H), 6.41 (t, 1H), 6.73 (d, 1H), 7.53 (m, 5H), 7.65 (d, 1H), 7.93 (s, 1H). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.11 (m, 2P), -21.58 (m, 2P).

Example 12

P^1, P^4-Di((4-bromophenyl)ethenocytidine 5'-) - tetraphosphate

Example 12 product was prepared from 50 mg P^1,P^4-Di(cytidine 5'-) tetraphosphate, tetrasodium salt and 2,4'-dibromoacetophenone, according to the general method of example 10. Yield= 20 mg (29%).

^{1}H NMR (D_2O, 300 MHz): δ 4.24 (m, 10H), 5.98 (d, 2H), 6.39 (d, 2H), 7.14 (m, 8H), 7.45 (m, 4H).). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.13 (m, 2P), -21.68 (m, 2P).

Example 13

P^1-((4-phenylphenyl)ethenocytidine 5'-) -P^4-(cytidine 5'-) tetraphosphate

Example 13 product was prepared from 50 mg P^1,P4-Di(cytidine 5'-) tetraphosphate, tetrasodium salt and 2-bromo-4'-phenylacetophenone, according to the general method of example 10. Yield= 15 mg (13%).

^{1}H NMR (D_2O, 300 MHz): δ 4.10 (m, 10H), 5.48 (d, 1H), 5.87 (m, 2H), 6.68 (d, 1H), 7.20 (m, 3H), 7.36 (m, 6H), 7.68 (m, 3H). ^{31}P NMR (D_2O, 121.47 MHz): δ -10.08 (m, 2P), -21.78 (m, 2P).

The products of examples 11-13 can be further derivatized according to the methods of Examples 1-8, to give bifunctional molecules that fall within the scope of the invention.

Example 14

2', 3'- phenylacetaldehyde acetal adenosine 5'-monophosphate

Adenosine 5'-monophosphate, free acid (10.0 g, 28.8 mmol) was dissolved in trifluoroacetic acid (50 mL) and phenylacetaldehyde, dimethylacetal (18.50 mL, 121 mmol) added. The reaction was stirred at ambient temperature for 3 hours, after which the trifluoroacetic acid was evaporated and the residue partitioned between 1 M sodium bicarbonate (80 mL) and ethyl acetate (40 mL). The layers were separated, and the product was isolated from the aqueous layer via C_{18} preparative HPLC. Yield= 7.50 g (59 %). The ammonium salt so obtained was converted to the mono-tributylammonium salt via treatment with a slight excess of tributylamine in aqueous N,N-dimethylformamide, followed by evaporation and drying.

^{1}H NMR (D_2O, 300 MHz): δ 3.06 (d, 2H), 3.86 (m, 2H), 4.39 (m, 1H), 4.91 (m, 1H), 5.18 (m, 1H), 5.36 (t, 1H), 5.63 (d, 1H), 7.23 (m, 5H), 8.09 (s, 1H), 8.20 (s, 1H). ^{31}P NMR (D_2O, 121.47 MHz): δ 2.17 (s).

Example 15

2', 3'- cinnamyl acetal adenosine 5'-monophosphate

Adenosine 5'-monophosphate, free acid (1.0 g, 2.88 mmol) was dissolved in 98% formic acid (5 mL) and cinnamaldehyde (1.14 g, 8.65 mmol) added. The reaction was stirred at ambient temperature for 3 hours, after which the formic acid was evaporated and the residue partitioned between 1 M sodium bicarbonate (25 mL) and ethyl acetate (20 mL). The

layers were separated, and the product was isolated from the aqueous layer via preparative HPLC. Yield= 0.202 g (15%).

^{1}H NMR (D_2O, 300 MHz): δ 3.97 (m, 2H), 4.50 (m, 1H), 5.04 (m, 1H), 5.29 (m, 1H), 5.65 (d, 0.4H), 5.86 (d, 0.6H), 6.24 (m, 2H), 6.87 (dd, 1H), 7.27 (m, 3H), 7.43 (m, 2H), 8.12 (d, 1H), 8.28 (d, 1H). ^{31}P NMR (D_2O, 121.47 MHz): δ 1.42 (d).

Example 16

2', 3'- phenylacetaldehyde acetal-6-N-phenylurea adenosine 5'-monophosphate (compound 22)

2',3'- phenylacetaldehyde acetal adenosine 5'-monophosphate, tributylammonium salt (prepared according to example 14, 1.0 g, 2.15 mmol) was dissolved in N,N-dimethylformamide (10 mL) and phenylisocyanate (1.17 g, 10.72 mmol) added. The reaction was heated at 35°C for 4 hrs, after which the solvent was removed and the residue partitioned between 1 M sodium bicarbonate (30 mL) and ethyl acetate (25 mL). The layers were separated and the product isolated from the aqueous layer via preparative HPLC. Yield = 0.85 g (68%).

^{1}H NMR (D_2O, 300 MHz): δ 2.97 (d, 2H), 3.81 (m, 2H), 4.31 (m, 1H), 4.78 (m, 1H), 4.98 (m, 1H), 5.23 (t, 1H), 5.63 (d, 1H), 6.74 (m, 1H), 6.96 (m, 4H), 7.19 (m, 5), 8.12 (s, 1H), 8.30 (s, 1H). ^{31}P NMR (D_2O, 121.47 MHz): δ 1.19 (s).

Example 17

2', 3'-cinnamyl acetal-6N-ethylurea adenosine 5'-monophosphate (compound 27)

Compound 27 was prepared according to example 16, starting with 2'3' cinnamyl acetal adenosine 5'-monophosphate (example 15) and substituting ethyl isocyanate for phenylisocyanate. Yield= 65%.

^{1}H NMR (D_2O, 300 MHz): δ 1.07 (t, 3H), 3.21 (q, 2H), 3.93 (m, 2H), 4.45 (m, 1H), 4.99 (m, 1H), 5.28 (m, 1H), 5.54 (d, 0.3H), 5.70 (d, 0.7H), 5.95 (m, 1H), 6.14 (m, 1H), 6.61 (dd, 1H), 7.14 (m, 5H), 8.29 (m, 2H). ^{31}P NMR (D_2O, 121.47 MHz): δ 1.93 (d).

Example 18

Inhibition of ADP-Induced Platelet Aggregation

Isolation of Platelets: Human blood was obtained from informed healthy volunteers. Blood was collected into one-sixth volume of ACD (2.5 g of sodium citrate, 1.5 g citric acid, and 2.5 g glucose in 100 ml dH_2O). Blood was centrifuged at 800 x g for 15 min at room temperature and the platelet-rich plasma removed and incubated for 60 min at 37 °C in the presence of 1 mM acetylsalicylic acid followed by centrifugation at 1000 x g for 10 min at room temperature. The platelet pellet was resuspended at a density of 2 x 10^8 cells/ml with HEPES-buffered Tyrode's solution (137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 3 mM NaH_2PO_4, 5 mM glucose, 10 mM HEPES pH 7.4, 0.2% bovine serum albumin, and 0.05 U/ml apyrase).

Aggregation Studies: ADP-induced platelet aggregation was determined by measuring the transmission of light through a 0.5 ml suspension of stirred (900 rpm) aspirin-treated washed platelets in a lumi-aggregometer at 37 °C (Chrono-Log Corp. Havertown, PA). The baseline of the instrument was set using 0.5 ml of Hepes-buffered Tyrode's solution. Prior to aggregation measurements, the platelet suspension was supplemented with 2 mM $CaCl_2$ and 1 mg/ml fibrinogen. Platelet aggregation was initiated by the addition of indicated concentrations of ADP or other agonists, and the light transmission continuously recorded for at least 8 min. When inhibitors of platelet aggregation were tested, platelets were incubated for 3-6 min in the presence of indicated concentrations of inhibitor before addition of ADP or other agonists, and the response recorded for at least 8 min. The potency of agonists and inhibitors of platelet aggregation was calculated from both, the rate of aggregation and the maximal extent of aggregation obtained for each determination by fitting the data to a four-parameter logistic equation using the GraphPad software package (GraphPad Corp. San Diego, CA).

The ability of $P2Y_{12}$ antagonists to inhibit platelet aggregation is presented in this application as the percent inhibition of the aggregation induced by a maximally effective concentration of ADP. When a broad range of concentrations of $P2Y_{12}$ antagonist was tested (usually from 1nM to 100 μM), an IC_{50} value was also obtained. IC_{50} values represent the concentration of antagonist needed to inhibit by 50% the aggregation elicited by a given concentration of ADP.

Example 19

Effects on Platelet Aggregation *In Vivo*

To evaluate the ability of these compounds to inhibit platelet aggregation *in vivo*, an experimental protocol similar to the method of R. G. Humphries et al. (Br. J. Pharmacol. 115:1110-1116, 1995) will be performed.

Surgical Preparation and Instrumentation: Male Sprague-Dawley rats are anesthetized. Body temperature is maintained at 37 ± 0.5°C with a heating lamp. Animals breathe spontaneously and a tracheotomy is performed to ensure a patent airway. A cannula containing heparinized saline is introduced into the left femoral artery and connected to a transducer to record blood pressure and heart rate. Cannulae containing non-heparinized saline are introduced into the left common carotid artery and left jugular vein for withdrawal of arterial blood samples and i.v. administration of compounds, respectively.

Experimental Protocol: Either compound or vehicle is administered to each animal as an infusion. Blood samples are taken immediately prior to the first infusion, at the end of each infusion and 20 min after cessation of the final infusion for measurement of platelet aggregation ex vivo. Immediately after sampling, ADP-induced platelet aggregation is measured in duplicate in 0.5 ml blood samples diluted 1:1 with saline and incubated at 37°C for 4 min. For the final minute of this period, cuvettes are transferred to a lumi-aggregometer and the sample stirred at 900 rpm. ADP (3 μM) is added in a volume of 20 μl and the aggregation response is recorded.

Example 20

Inhibition of Thrombus Formation in Anesthetized Rats

To evaluate the effect of these compounds on thrombus formation *in vivo*, the following experimental protocol will be performed.

Rats (CD-1; male; approximately 350 grams; Charles River, Raleigh, NC), are anesthetized with sodium pentobarbital (70 mg/kg i.p.). The abdomens are shaved and a 22 gauge intravenous catheter is inserted into a lateral tail vein. A midline incision is made and the intestines are wrapped in saline-soaked gauze and positioned so the abdominal aorta is accessible. The inferior vena cava and abdominal aorta are carefully isolated and a section (approx. 1 cm) of the abdominal aorta (distal to the renal arteries proximal to the bifurcation) is dissected. All branches from the aorta in this section are ligated with 4-0 silk suture. A 2.5 mm diameter flow probe connected to a Transonic flow meter is placed on the artery and a

baseline (pre-stenosis) flow is recorded. Two clips are placed around the artery decreasing the vessel diameter by approximately 80%. A second baseline flow measurement is taken (post-stenosis) and the hyperemic response is tested. Animals are then treated with either compound or saline i.v., via tail vein catheter. Thrombosis is induced five minutes after treatment by repeated external compressions of the vessel with hemostatic forceps. Two minutes post-injury, the vessel compressions are repeated and a 10 minute period of flow monitoring is started. Animals are monitored continuously for a minimum of the first ten minutes post-injury. After twenty minutes (post-injury), a flow measurement is repeated and the animals are euthanized. The section of the aorta that includes the injured section is harvested and placed in 10% formalin for possible histologic evaluation.

Example 21

Inhibition of Thrombus Formation in Anesthetized Dogs

To evaluate the effect of these compounds on dynamic thrombus formation *in vivo*, the following experimental protocol similar to the method of J. L. Romson et al. (*Thromb. Res.* 17:841-853, 1980) will be performed.

Surgical Preparation and Instrumentation: Briefly, purpose-bred dogs are anesthetized, intubated and ventilated with room air. The heart is exposed by a left thoracotomy in the fifth intercostal space and suspended in a pericardial cradle. A 2-3 cm segment of the left circumflex coronary artery (LCCA) is isolated by blunt dissection. The artery is instrumented from proximal to distal with a flow probe, a stimulation electrode, and a Goldblatt clamp. The flow probe monitors the mean and phasic LCCA blood flow velocities. The stimulation electrode and its placement in the LCCA and the methodology to induce an occlusive coronary thrombus have been described previously (J. K. Mickelson et al., *Circulation* 81:617-627, 1990; R. J. Shebuski et al., Circulation 82:169-177, 1990; J. F. Tschopp et al., Coron. Artery Dis. 4:809-817, 1993).

Experimental Protocol: Dogs are randomized to one of four treatment protocols (n=6 per treatment group) in which the control group receives saline i.v. and the three drug-treated groups are administered compound i.v. Upon stabilization from the surgical interventions, dogs receive either saline or compound. After approximately 30 minutes, an anodal current is applied to the LCCA for 180 min. The number and frequency of cyclic flow variations (CFV) that precede formation of an occlusive thrombus are recorded. These cyclic phenomena are caused by platelet thrombi that form in the narrowed lumen as a result of platelet aggregation

(J. D. Folts et al., Circulation 54:365-370, 1976; Bush et al., Circulation 69:1161-1170, 1984). Zero flow in the LCCA for a minimum of 30 minutes indicates a lack of antithrombotic efficacy (L.G. Frederick *et al., Circulation* 93:129-134, 1996).

Example 22

ADP-Induced Aggregation of Different Compounds

Different compounds were tested for their inhibition of ADP-induced aggregation and their IC_{50} according to the protocols in Example 18; the results are shown in Figure 1. The bar graphs in the figure illustrate the effect of 100 μM concentration of the compound on ADP-induced platelet aggregation, and the data are expressed as % inhibition of the ADP response.

Figure 1 shows the structure and abbreviated name of each compound and its activity. Where hydrogens are understood to be present, they have been omitted for the sake of simplicity. For example, for the first structure of the figure, it is implied that there are hydrogens at the 3- position of the pyrimidine ring, at the 3'- position of the ribose on the oxygen, and on the nitrogen of the carbamate at the 2'- position of the ribose. In addition, as disclosed within the scope of the present invention, it is implied that the oxygens that are not doubly bonded to the phosphorous atoms are either present in the ionized form as salts with a counterion, or are bonded to a hydrogen atom. For simplicity, some of the structures in the figure are portrayed in the salt form, but this should not be interpreted as excluding the possibility that hydrogens could be present instead.

Several parent compounds, Up4U, Ip4U, Up3U, and Cp4U, without modifications on the furanose hydroxyl groups, have been included at the end of the figure to illustrate the utility of the present invention. However, these unmodified parent compounds do not inhibit the ADP-induced aggregation and are not within the scope of the present invention.

Although the invention has been described with reference to the presently preferred embodiments, it should be understood that various modifications could be made without departing from the scope of the invention.